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(54) Title: **HUMAN INTERLEUKIN-1 RECEPTOR ACCESSORY PROTEIN**

(57) Abstract

This invention is directed to polynucleotides encoding human IL-1 receptor accessory protein, isolated IL-1 receptor accessory protein, and antibodies to IL-1 receptor accessory protein. This protein is particularly useful to prevent inflammation due to the action of IL-1.

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- 1 -

Human Interleukin-1 Receptor accessory protein

The present invention relates generally to cytokine receptors, and more specifically to accessory proteins of interleukin 1 receptors.

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Interleukin 1 (IL-1) is a polypeptide hormone that acts on a variety of cell types and has multiple biological properties (Dinarello, Blood 77: 1627, 1991). IL-1 is a major mediator of inflammatory and immune responses. Therefore, regulation of IL-1 activity provides a 10 means of controlling and modulating these responses.

Two species of IL-1 have been characterized, interleukin 1 α (IL-1 α) and interleukin 1 β (IL-1 β), both of which are referred to herein as IL-1. The biological activities produced by IL-1 are 15 mediated by binding to specific plasma membrane receptors, termed the Type I and Type II IL-1 receptors. The IL-1 receptors (IL-1R's) are transmembrane proteins with extracellular domains of about 300 amino acids, and are members of the immunoglobulin superfamily of molecules (Sims et al., Science 241: 585, 1988; Sims et al., Proc. Natl. Acad. Sci. USA 86: 8946, 1989; McMahan et al., EMBO J. 10: 2821, 20 1991). Both IL-1 species bind to each of these receptors and compete completely with each other for binding.

It has been assumed that the Type I IL-1R encodes the entire 25 functional IL-1 receptor. Experiments with the cloned Type I IL-1R indicated that when this receptor protein was transfected and expressed in Chinese hamster ovary cells, it was sufficient to bind IL-1 and to transduce the IL-1 signal (Curtis et al., Proc. Natl. Acad. Sci. USA 86: 3045, 1989). The presence of an accessory protein 30 endogenous to the hamster cells was not determined in these studies. It had been suggested that the Type II IL-1R represented an accessory chain of the IL-1R (Solari, Cytokine 2: 21, 1990). However, more recent studies have shown that the Type II IL-1R is unlikely to function as a signal-transducing accessory protein, and that it acts

- 2 -

instead as a decoy receptor to bind excess IL-1 and regulate its activity (Colotta et al., *Science* 261: 472, 1993).

Since IL-1 binding to the IL-1 receptor mediates the biological effects of IL-1, an understanding of the mechanism of receptor binding and activation is important for regulating IL-1's activities. Affinity crosslinking and binding studies with labelled IL-1 have shown that the IL-1 receptor exists as a complex of multiple proteins that can bind IL-1 with different affinities (Lowenthal and MacDonald, *J. Exp. Med.* 164: 1060, 1986; Bensiman et al., *J. Immunol.* 143:1168, 1989; McMahan et al., *EMBO J.* 10:2821, 1991). A murine monoclonal (mAb) 4C5 has been described that recognizes a 90 kDa protein on murine cells that is associated with IL-1R and is required for signal transduction and biological activity (Powers et al., AAI meeting, Denver, CO, May 21-25, 1993). It was not known if an equivalent protein existed on human cells, or what biological function, if any, was associated with such a protein.

Prior to the present invention, efforts to identify a human IL-1R accessory protein or to clone and express genes encoding this protein have been significantly impeded by lack of purified protein, lack of an antibody that recognizes this protein, and inability to identify cells that express large amounts of this protein and its mRNA. Even the murine accessory protein had not been obtained in sufficient amounts to use in efforts to identify the corresponding human accessory protein. Murine cell lines known to express the accessory protein did so only in amounts (~1000 molecules/cell) too low to purify sufficient protein for obtaining unambiguous amino acid sequence information. There was no mAb known to recognize a human homologue of the 4C5 target protein (the murine accessory protein). In addition, binding to IL-1 was not known to be an effective screen for identifying a human accessory protein, since it is known that many accessory proteins do not bind ligand or bind with very low affinity (Hibi et al., *Cell* 63: 1149, 1990; Takeshita et al., *Science* 257: 379, 1992).

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This invention makes available for the first time purified human IL-1 receptor accessory protein which can be used to regulate the effects of IL-1. The addition of soluble accessory protein inhibits the

effect of IL-1 on the cells. Hence, an aspect of the invention is the treatment of pathological conditions caused by excess activity of cells responding to IL-1 by adding an amount of soluble human IL-1R accessory protein (IL-1R AcP) sufficient to inhibit activation of cells 5 by IL-1. This methodology can also be modified, and the soluble accessory protein can be used as a screening agent for pharmaceuticals.

Briefly, a pharmaceutical which works as an IL-1 antagonist can 10 do so by blocking the interaction of IL-1 with the IL-1R AcP. The presence of IL-1R AcP in a cell membrane is necessary to permit IL-1 to interact effectively with the IL-1 receptor complex (by effective interaction is meant binding to the receptor complex so as to initiate a biological response). The IL-1 receptor complex includes the Type I or 15 Type II IL-1 receptor in association with the IL-1R AcP (additional proteins may also be part of the complex). Adding soluble IL-1R AcP inhibits this interaction by allowing IL-1 or the IL-1 receptor to interact with the soluble protein instead of IL-1R AcP on the cell surface, thus reducing the biological response caused by IL-1. 20 Antibodies to the IL-1R AcP of this invention similarly inhibit the biological response of cells to IL-1. By binding to the IL-1R AcP, antibodies prevent IL-1 from interacting effectively with the IL-1 receptor. By blocking IL-1R AcP, these antibodies inhibit the binding 25 of IL-1 to the IL-1 receptor complex, which depends on interaction with IL-1R AcP. IL-1R AcP will inhibit IL-1 interaction with the IL-1 receptor, thus preventing activation of IL-1 responsive cells and decreasing the inflammatory response. One may also use the purified IL-1R AcP to screen a potential pharmaceutical. If the pharmaceutical blocks IL-1 binding to the IL-1R AcP, it will be an effective IL-1 30 antagonist.

The present invention provides polynucleotides which encode IL-1 receptor accessory proteins or active fragments thereof, preferably, the polynucleotides are selected from a group consisting 35 of (a) polynucleotides, preferably cDNA clones, having essentially a nucleotide sequence derived from the coding region of a native IL-1R AcP gene, such as shown in Figure 15 [SEQ ID NO. 1]; (b) poly-nucleotides capable of hybridizing to the cDNA clones of (a) under

moderately stringent conditions and which encode IL-1R AcP or fragments thereof; and (c) polynucleotides which are degenerate as a result of the genetic code to the DNA sequences defined in (a) and (b) and which encode IL-1R AcP molecules or fragments thereof.

- 5 Particularly preferred compounds are the polynucleotides which encode human IL-1 receptor accessory proteins, e. g. the polynucleotides encoding the amino acid sequence [SEQ ID NO:3] or an active fragment thereof, especially a polynucleotide having the sequence [SEQ ID NO:1]. Especially preferred compounds encode
10 soluble IL-1 receptor accessory proteins, e. g. human soluble IL-1 receptor accessory proteins having for example the amino acid sequence [SEQ ID NO:9]. The polynucleotide [SEQ ID NO:7] codes for a human soluble IL-1 receptor accessory protein. Also part of this invention are the antisense polynucleotides of the above compounds.

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The present invention also provides vectors and suitable host cells, preferably expression vectors comprising the DNA sequences defined above, recombinant IL-1R AcP produced using the expression vectors, and a method for producing the recombinant accessory
20 protein molecules utilizing the expression vectors.

The present invention makes available IL-1 receptor accessory proteins and active fragments thereof, encoded by polynucleotides as defined above. Preferred compounds are human IL-1 receptor
25 accessory proteins, preferably a protein having the amino acid sequence [SEQ ID NO:3]. Especially preferred are soluble human IL-1 receptor accessory proteins, e. g. having the amino acid sequence [SEQ ID NO:9]. Also part of this invention are IL-1R AcP proteins carrying one or more side groups which have been modified.

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The present invention also provides antibodies to IL-1R AcP. These antibodies bind specifically to the human IL-1 receptor accessory protein and prevent activation of the IL-1 receptor complex by IL-1. The preferred antibodies have a binding affinity to the IL-1 receptor accessory complex of from about K_D 0.1 nM to about K_D 10 nM and are for example monoclonal antibodies or derivatives thereof.

Also part of this invention are pharmaceutical compositions which comprise an antisense polynucleotide, a IL-1 receptor accessory protein or an antibody as described above. These pharmaceutical compositions may include one or more other cytokine 5 antagonists.

The invention also provides a process for the preparation of an IL-1 receptor accessory protein comprising the steps of (a) expressing a polypeptide encoded by an above mentioned polynucleotide in a 10 suitable host, (b) isolating said IL-1 receptor accessory protein, and (c) if desired, converting it in an analogue wherein one or more side groups are modified. Moreover, the invention includes a process for the preparation of an IL-1 receptor accessory protein antibody comprising the steps of (a) preparation of a hybridoma cell line 15 producing a monoclonal antibody which specifically binds to the IL-1 receptor accessory protein and (b) production and isolation of the monoclonal antibody. Corresponding polyclonal antibodies may be produced using known methods.

20 The above mentioned compounds are useful as therapeutically active substances, e. g. for use in the treatment of inflammatory or immune responses and/or for regulating and preventing inflammatory or immunological activities of Interleukin-1. Especially, these compounds are useful in the treatment of acute or chronic 25 diseases, preferably rheumatoid arthritis, inflammatory bowel disease, septic shock, transplant rejection, psoriasis, asthma and Type I diabetes or, in the treatment of cancer, preferably acute and chronic myelogenous leukemia.

30 As used herein, IL-1 includes both IL-1 α and IL-1 β , and IL-1 receptor includes Type I and Type II IL-1 receptors, unless otherwise specifically indicated.

35 BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Equilibrium Binding of [125 I]-4C5 to Murine EL-4 Cells at Room Temperature. EL-4 cells (1.5×10^6 cells) were

incubated for 2 hrs at room temperature with increasing concentrations of [¹²⁵I]-4C5 in the absence (o) or presence (▽) of 100 nM unlabeled 4C5. Total (o) and non-specific (▽) cell bound radioactivity were determined as described in Example 1. Specific binding of [¹²⁵I]-4C5 (•) was calculated by subtracting non-specific binding from total binding. 1A. Binding of EL-4 cells incubated with [¹²⁵I]-4C5. 1B. Analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model.

Figure 2. Equilibrium binding of [¹²⁵I]-4C5 to Murine 70Z/3 Cells. 70Z/3 cells (1.5×10^6) were incubated for 2 hrs at room temperature with increasing concentrations of [¹²⁵I]-4C5 in the absence (o) or presence (▽) of 100 nM unlabeled 4C5. Total (o) and non-specific (▽) cell bound radioactivity were determined as described in Example 1. Specific binding of [¹²⁵I]-4C5 (•) was calculated by subtracting non-specific binding from total binding. 2A. Binding of 70Z/3 cells incubated with [¹²⁵I]-4C5. 2B. Analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model.

Figure 3. Inhibition of Human [¹²⁵I]-IL-1 Binding to IL-1 Receptor on 70Z/3 Cells by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition assays were performed as described in Example 1. The data are expressed as the percent inhibition of [¹²⁵I]-IL-1 binding in the presence of the indicated concentrations of antibody when compared to the specific binding in the absence of antibody. Proteins are human IL-1 α (H-alpha) and human IL-1 β (H-beta).

Figure 4. Inhibition of Human [¹²⁵I]-IL-1 Binding to IL-1 Receptor on EL-4 Cells by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition assays were performed as described in Example 1. The data are expressed as the percent inhibition of [¹²⁵I]-IL-1 binding in the presence of the indicated concentrations of antibody when

compared to the specific binding in the absence of antibody. Proteins are human IL-1 α (H-alpha) and human IL-1 β (H-beta).

Figure 5. Isolation of Two Proteins of 90 and 50 kDa from a
5 Solubilized Extract of EL-4 Cells by 4C5 Affinity Chromatography.
Proteins were partially purified from a detergent extract of EL-4 cells
by lentil lectin affinity chromatography followed by affinity
chromatography on a matrix containing either an anti-Type I IL-1R
antibody (7E6), murine IL-1 α (Ma) or anti-accessory protein antibody
10 (4C5) as described in Example 1. Proteins in the detergent extract of
EL-4 cells were also directly purified on a 4C5 affinity matrix (4C5).
The proteins eluted from the columns were separated by SDS-PAGE,
transferred to nitrocellulose and probed with [¹²⁵I]-4C5. The
molecular sizes indicated in the margins were estimated from
15 molecular weight standards (Amersham Prestained Standards) run in
parallel lanes. Exposure time was 1 day.

Figure 6. Inhibition of IL-1 Induced Splenic B Cell
Proliferation by Monoclonal Antibodies 4C5, 4E2 and 35F5. Inhibition
20 assays were performed as described in Example 1. The data are
expressed as the incorporation of ³H-thymidine (CPM) by B cells in
the presence of the indicated concentrations of antibody when
compared to the incorporation in the absence of antibody. Proteins
are: 6A. human IL-1 α (IL-1 α) and 6B. human IL-1 β (IL-1 β).
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Figure 7. Inhibition of IL-1 Induced Proliferation of D10.G4.1
Helper T-cells by Monoclonal Antibodies 4C5 and 35F5 and Human
IL-1ra. Inhibition assays were performed as described in Example 1.
The data are expressed as the incorporation of ³H-thymidine (CPM)
30 by D10 cells in the presence of the indicated concentrations of
antibody and IL-1ra when compared to the incorporation in the
absence of antibody or IL-1ra. Proteins are: 7A. human IL-1 α , 7B.
human IL-1 β .

35 Figure 8. Inhibition of IL-1 Induced Kappa Light Chain
Expression by 70Z/3 Cells: Effect of Monoclonal Antibodies 4C5, 4E2
and 35F5. The induction of kappa light chain expression and
inhibition with the antibodies was as described in Example 1. The

data are expressed as the percent of cells expressing kappa light chain in the presence of the indicated concentrations of antibody when compared to the percent of cells in the absence of antibody. Proteins are human IL-1 α (IL-1 α) and human IL-1 β (IL-1 β).

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Figure 9. Inhibition of IL-1 Induced Serum IL-6 in C57BL/6 Mice by Monoclonal Antibodies 4C5 and 35F5. Mice were pretreated with the monoclonal antibody at 4 hrs and 10 mins prior to subcutaneous injection of human IL-1 α (alpha) or human IL-1 β (beta) (0.03 μ g). Two hours after the IL-1 administration, the serum IL-6 concentration was determined as described in Example 1. Mab X-7B2 is a control antibody.

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Figure 10. Nucleotide Sequence and Deduced Amino Acid Sequence of Murine IL-1R AcP. 10A. The nucleotide sequence of the opening reading frame of murine IL-1R AcP cDNA clone E2-K is shown. The top strand is the coding sequence [SEQ ID NO:4]. 10B. The amino acid sequence of murine IL-1R AcP as deduced from the coding sequence shown in Figure 10A is shown [SEQ ID NO:6]. The signal peptide cleavage site is predicted to occur after Ala -1, resulting in a 550 amino acid mature protein that extends from Ser 1 to Val 550. The cleavage site has been confirmed by NH₂-terminal sequence analysis of purified natural muIL-1R AcP (Example 10). The predicted transmembrane domain extends from Leu 340 through Leu 20

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363.

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Figure 11. Immunoprecipitation of Recombinant MuIL-1R AcP from Transfected COS cells with mAbs 4C5 and 2E6. COS cells were transfected by electroporation with either pEF-BOS/muIL-1R AcP or pEF-BOS alone (mock). Transfected cells were metabolically labelled with [³⁵S]Met as described (Example 8). Labelled transfectants were solubilized with RIPA buffer and immunoprecipitated with either mAb 4C5 or 2E6 (see Table 2) as described (Example 8). Both mAbs immunoprecipitated labelled protein from COS cells transfected with pEF-BOS/muIL-1R AcP which migrated as a broad band between 70-90 kDa. No labelled protein was detected in this size range from mock transfected COS cells. A higher molecular weight species (>200 kDa) is present in both mock and muIL-1R AcP transfected COS cells.

Figure 12. Equilibrium Binding of [¹²⁵I]-Labeled 4C5 and IL-1 to Murine Recombinant IL-1R AcP Expressed in COS-7 Cells. Cells (4-8 x 10⁴) transfected with an IL-1R AcP expression plasmid [COS(AcP)] or control plasmid [COS(PEF-BOS)] were incubated for 3 hrs at 4°C with increasing concentrations of [¹²⁵I]-4C5 or [¹²⁵I]-IL-1 α in the absence (Total) or presence (Non-Specific) of 100 nM unlabeled 4C5 or 50 nM unlabeled IL-1 α . Total (Total) and non-specific (Non-Specific) cell bound radioactivity were determined as described in Example 1. Specific binding of [¹²⁵I]-4C5 (Specific) and [¹²⁵I]-IL-1 α (Specific) were calculated by subtracting non-specific binding from total binding. The binding of [¹²⁵I]-IL-1 α to COS cells transfected with the control plasmid [COS(PEF-BOS)] showed that Cos-7 cells naturally express approximately 600 high affinity binding sites for IL-1 α . The right hand panel shows analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213, 1985) with a single-site model. 12A. Binding of COS(AcP) cells incubated with [¹²⁵I]-4C5 12B. Scatchard plot of 12A data. 12C. Binding of COS(AcP) cells incubated with [¹²⁵I]-IL-1 α 12D. Scatchard plot of 12C data. 12E. Binding of [COS(PEF-BOS)] cells incubated with [¹²⁵I]-IL-1 α . 12F. Scatchard plot of 12E data.

Figure 13. Equilibrium Binding of [¹²⁵I]-Labeled 35F5 and IL-1 to Murine Recombinant Type I IL-1R Expressed in COS-7 Cells. Cells (4-8 x 10⁴) transfected with an Type I IL-1R expression plasmid [COS(Mu-IL-1R)] were incubated for 3 hrs at 4°C with increasing concentrations of [¹²⁵I]-35F5 or [¹²⁵I]-IL-1 α and [¹²⁵I]-IL-1 β in the absence (Total) or presence (Non-Specific) of 100 nM unlabeled 35F5 or 50 nM unlabeled IL-1. Total (Total) and non-specific (Non-Specific) cell bound radioactivity were determined as described in Example 1. Specific binding of [¹²⁵I]-35F5 (Specific) and [¹²⁵I]-IL-1 α or IL-1 β (Specific) were calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949) as determined by Ligand (Munson and Rodbard, Anal. Biochem. 107: 220, 1980; McPherson, J. Pharmacol. Methods 14: 213,

1985) with a single-site model. 13A. Binding of [COS(Mu-IL-1R)] cells incubated with [¹²⁵I]-35F5. 13B. Scatchard plot of 13A data. 13C. Binding of [COS(Mu-IL-1R)] cells incubated with [¹²⁵I]-IL-1 β . 13D. Scatchard plot of 13C data. 13E. Binding of [COS(Mu-IL-1R)] cells incubated with [¹²⁵I]-IL-1 α . 13F. Scatchard plot of 13E data.

Figure 14. Construction of Full-length cDNA Clone of Human IL-1R AcP. Schematic representations of the structures of the human IL-1R AcP cDNA inserts in clones #3 and #6 are shown in the upper portion of the figure. Clone #3 contains 5' noncoding sequences, the initiating ATG codon, and a significant portion of the coding region. Clone #6 overlaps with clone #3, containing most of the coding region, the TGA stop codon, and 3' noncoding sequences. The 846 bp *Xba*I/*Bst*XI fragment from clone #3 and the \approx 2700 bp *Bst*XI/*Xba*I fragment from clone #6 were isolated and ligated into the expression vector pEF-BOS as described (Examples 12 and 13). A schematic representation of the resulting cDNA encoding full-length human IL-1R AcP is shown on the bottom line.

Figure 15. Nucleotide Sequence of Human IL-1R AcP. The nucleotide sequence of the open reading frame in the full-length human IL-1R AcP cDNA (Example 13, Figure 14) is shown. The top strand is the coding sequence [SEQ ID NO:1].

Figure 16. Amino Acid Sequence of Human IL-1R AcP. The amino acid sequence of human IL-1R AcP as deduced from translation of the nucleotide sequence in Figure 15 is shown [SEQ ID NO:3]. The signal peptide cleavage site is predicted to occur after Ala-1, resulting in the production of a 550-amino acid mature protein that extends from Ser1 to Val550. The predicted transmembrane domain extends from Leu340 to Leu363.

Figure 17. IL-1 Induction of IL-6 Production in MRC-5 Cells: Inhibition by IL-1 Receptor Antagonist and Anti-Type I IL-1 Receptor Antibody 4C1. Human embryonic lung fibroblast MRC-5 cells (5×10^4 cells; ATCC# CCL-171) were plated into 24-well cluster dishes (No. 3524; Costar) for 24 hrs at 37°C in a humidified incubator. After the 24 hr period, the cells were pretreated with increasing concentrations of either IL-1 receptor antagonist (IL-1RA; 10^{-2} to

10³ pM), anti-Type I IL-1 receptor antibody 4C1 (10⁻⁴ to 10¹ µg/ml) or nothing for 1 hr at 37°C. At the end of 1 hr, either 5 pM or 100 pM human IL-1β was added and the incubation continued for 24 hrs at 37°C. At the end of the incubation period, 100 µl of cell supernatent
5 was removed from each well and assayed for IL-6 concentration by the Quantikine Human IL-6 Assay Kit (R & D Systems). The data are expressed as the concentration (pg/ml) of IL-6 secreted from the MRC-5 cells in presence of either IL-1β alone or in the presence of IL-1β plus inhibitor. The effect of increasing concentrations of tumor
10 necrosis factor-α (TNFα) on the stimulation of IL-6 secretion from MRC-5 cells was also determined. TNFα was less potent (~500-fold) than IL-1β in stimulating IL-6 secretion from these cells and appeared to be partially dependent on an autocrine secretion of IL-1 by these cells. 17A shows data for IL-1β, TNFα, and inhibition by IL-15
17B shows data for inhibition by mAb 4C1.

Figure 18. Nucleotide Sequence of the Soluble Human IL-1R AcP. The nucleotide sequence of the soluble human IL-1R AcP cDNA is shown. The top strand is the coding sequence [SEQ ID NO:7].

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Figure 19. Amino Acid Sequence of the Soluble Human IL-1R AcP. The amino acid sequence of soluble human IL-1R AcP as deduced from translation of the nucleotide sequence in Figure 18 is shown [SEQ ID NO:9].

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The present invention is directed to an isolated polynucleotide that encodes a IL-1R AcP (IL-1R AcP) or an active fragment of a IL-1R AcP (i.e. capable of inhibiting the ability of IL-1 to bind to or otherwise activate the IL-1 receptor), in particular a human or
30 murine IL-1R AcP. Examples of such a polynucleotide are the DNA polynucleotide having the sequence [SEQ ID NO: 1], and the DNA polynucleotide encoding the human IL-1R AcP which has the amino acid sequence [SEQ ID NO: 3]. The polynucleotides of this invention may be used as intermediates to produce the protein IL-1R AcP as
35 described below. This protein is useful in treatment of conditions related to IL-1 inflammatory activity. The polynucleotides may themselves be used in treatment by known antisense modalities.

The invention is also directed to IL-1 receptor accessory protein (IL-1R AcP) isolated free of other proteins, or an isolated active fragment of IL-1R AcP. The IL-1R AcP of this invention is a protein or active fragment which inhibits the ability of IL-1 to bind to or 5 otherwise activate the IL-1 receptor.

Part of this invention is a method of obtaining human IL-1R AcP, which method uses as intermediates the following compounds: polynucleotides encoding murine IL-1RAcP, murine IL-1R AcP, 10 antibodies to murine IL-1R AcP, and polynucleotides encoding human IL-1R AcP. From polynucleotides encoding human IL-1R AcP, soluble human IL-1R AcP and antibodies thereof can be obtained. The critical first intermediate for this invention is the isolation of mAbs for the murine IL-1R accessory protein. These mAbs are obtained by 15 immunization with a partially purified preparation of solubilized crosslinked IL-1 α /IL-1R complex from murine 70Z/2 pre-B cells (described in Example 1). The use of the crosslinked ligand-receptor complex was uniquely suitable, since the accessory protein could only be purified as a result of its interaction in such a complex. One of 20 these mAbs (4C5) was then used to isolate a cDNA encoding the murine IL-1R AcP. This murine cDNA was used to obtain a partial genomic clone of the human homologue. A probe derived from the partial genomic clone was then used to isolate the full-length cDNA for human IL-1R AcP.

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As used herein, "polynucleotide" refers to an isolated DNA or RNA polymer, in the form of a separate molecule or as a component of a larger DNA or RNA construct, which has been derived from DNA or RNA isolated at least once in substantially pure form, i.e., free of 30 contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading 35 frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3'

from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

These polynucleotides, e. g. DNA, include those containing one or 5 more of the above-identified DNA sequences and those sequences which hybridize under stringent hybridization conditions (see, T. Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory (1982), pp. 387 to 389) to the DNA sequences. An example of one such stringent hybridization condition is hybridization 10 at 4 x SSC at 65°C, followed by a washing in 0.1 x SSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50 % formamide, 4 x SSC at 42°C.

Polynucleotides which hybridize to the sequences for IL-1R AcP 15 under moderate hybridization conditions and which code on expression for IL-1R AcP peptides having IL-1R AcP biological properties also encode novel IL-1R AcP polypeptides. Examples of such non-stringent hybridization conditions are 4 x SSC at 50°C or hybridization with 30 - 40 % formamide at 42°C. Additional 20 hybridization conditions are mentioned in Example 11. For example, a DNA sequence which shares regions of significant homology, e. g. sites of glycosylation or disulfide linkages, with the sequences of IL-1R AcP and encodes a protein having one or more IL-1R AcP biological properties clearly encodes a IL-1R AcP polypeptide even if such a 25 DNA sequence would not stringently hybridize to the IL-1R AcP sequences.

Polynucleotides of this invention were obtained as described in Examples 7-13 by expressing murine cDNA in eucaryotic cells and 30 screening cell-surface proteins using assays described in Example 7. A murine cDNA clone was identified which results in the expression of a protein immunoreactive with mAb 4C5. This cDNA clone was used to obtain the homologous human genomic clone. Briefly, human genomic DNA was screened with the intermediate murine IL-1R AcP probe 35 obtained from mouse cells in Example 7. Clones were isolated and sequenced as described. The partial human genomic clones were then used as intermediates to screen a human cDNA library and clones

were isolated and sequenced as described to obtain full-length polynucleotides of this invention encoding human IL-1R AcP.

A specific polynucleotide of this invention has the sequence [SEQ ID NO: 1]. Another polynucleotide of this invention encodes the human IL-1R AcP having the amino acid sequence [SEQ ID NO: 3]. Any polynucleotide capable of encoding the amino acid sequence of IL-1R AcP, or specifically [SEQ ID NO: 3] is part of this invention. Another polynucleotide of invention has the sequence [SEQ ID NO: 4].

10

Also part of this invention is a polynucleotide encoding an active fragment of IL-1R AcP. Such polynucleotides are fragments of the polynucleotides provided above (fragmented by known methods such as restriction digestion or shearing) which, when expressed by conventional methods, produce proteins that block IL-1 activity in an IL-1 assay described below. A polynucleotide encoding a soluble IL-1R AcP is a preferred fragment of this invention. An example of such a polynucleotide has the sequence [SEQ ID NO:7].

20 Polynucleotides encoding the IL-1R AcP and its active fragments are useful as intermediates from which IL-1R AcP and its active fragments are obtained. In addition, these polynucleotides are useful as antisense therapeutics which block the production of IL-1R AcP. Antisense therapeutics are used as described in Akhtar and Ivinston, 25 Nature Genetics 4:215, 1993. RNA or DNA polynucleotides both have these utilities. Antisense polynucleotides which are complementary to [SEQ ID NO:1] or to a fragment of this sequence are part of this invention. Such polynucleotides may be obtained by known methods such as DNA or RNA synthesis to produce a complementary sequence. 30 Thus, any sequence from the polynucleotides of this invention which is capable of hybridizing to DNA or RNA encoding IL-1R AcP under moderately stringent conditions known in the art and which when so hybridized prevents the synthesis of IL-1R AcP is also part of this invention.

35

This invention includes vectors which contain the polynucleotides described herein which encode IL-1R AcP or an active fragment. Any vector known in the art may be used in this capacity,

such as plasmids, phagemids, viral vectors, cosmids and other vectors. The polynucleotides are inserted in the vectors by methods well known in the art of recombinant DNA technology. Expression vectors are a particular example of vectors.

5

As used herein, "expression vector" refers to a vector such as plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or 10 coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in various eukaryotic expression systems preferably include a signal sequence enabling extracellular secretion of translated protein by a 15 host cell. Alternatively, where recombinant protein is expressed without a signal or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

20

Also part of this invention are host cells containing expression vectors containing polynucleotides of this invention, which express IL-1R AcP or active fragments. The polynucleotides are inserted into vectors containing transcriptional regulatory sequences to form 25 expression vectors. These expression vectors are then inserted into host cells by transfection, infection, electroporation, or other well-known methods. Such host cells are capable of producing protein from the expression vectors inserted therein. Other host cells, e.g. yeast, Chinese hamster ovary cells, bacterial cells, can be utilized with the 30 appropriate and suitable expression vectors.

As noted above, this invention is also directed to IL-1 receptor accessory protein (IL-1R AcP) isolated free of other proteins, or an active fragment of IL-1R AcP. The IL-1R AcP of this invention is a 35 protein or active fragment which inhibits the ability of IL-1 to bind to or otherwise activate the IL-1 receptor, especially the Type I IL-1 receptor. Inhibiting activation of the human IL-1 receptor is accomplished by the human IL-1R AcP or active fragments, and has

various effects, in particular reducing inflammation. Thus by means of the IL-1R AcP or active fragment, it is possible to inhibit IL-1 activation of cells and thereby to reduce or alleviate the symptoms associated with inflammation.

5

Active fragments of IL-1R AcP may be obtained by conventional methods for obtaining protein fragments. For example, DNA of this invention may be fragmented by restriction digest or shearing and expressed in host cells by conventional methods to provide fragments 10 of IL-1R AcP. Fragments of the IL-1R AcP may also be obtained by proteolysis of the IL-1R AcP of this invention. Active fragments of this invention are determined by screening for activity using IL-1 assays described below.

15

Soluble IL-1R AcP is an IL-1R AcP fragment of this invention in which deletions of the COOH-terminal sequences result in secretion of the protein into the culture medium. The soluble IL-1R AcP corresponds to all or part of the extracellular region of the IL-1R AcP. Methods for elucidating the COOH terminals and extracellular regions 20 of proteins are well known. The resulting protein preferably retains its ability to interact with IL-1 or the Type I and Type II IL-1R's. Particularly preferred sequences include those in which the transmembrane region and intracellular domain of the IL-1R AcP are deleted or substituted to facilitate secretion of the accessory protein 25 into the culture medium. The soluble IL-1R AcP may also include part of the transmembrane region, provided that the soluble IL-1R AcP is capable of being secreted from the cell. Soluble IL-1R AcP is obtained as described in Examples 14 and 15. A specific soluble IL-1R AcP of this invention has the sequence [SEQ ID NO:9].

30

A preferred example of IL-1R AcP has the amino acid sequence [SEQ ID NO: 3]. The amino acid sequence of the IL-1R AcP as deduced from the cDNA sequence [SEQ ID NO: 1] is shown in Figure 16. Any IL-1R AcP which affects IL-1 binding as described above, is included 35 in this invention, such as an analogue having the sequence of [SEQ ID NO: 3], in which one or more side groups have been modified in a known manner, by attachment of compounds such as polyethylene glycol, or by incorporation in a fusion protein (with other protein

- sequences such as immunoglobulin sequences), for example, or proteins whose activity has otherwise been maintained or enhanced by any such modification. Also included are proteins which inhibit IL-1 binding to the IL-1 receptor and have essentially the sequence
- 5 [SEQ ID NO:3] with one or more amino acids added, deleted, or substituted by known techniques such as site-directed mutagenesis. The change in amino acids is limited and conservative so as to maintain the identity of the protein as an IL-1R AcP with all or part of its activity as described, or enhanced activity. Means for
- 10 determining IL-1 inhibiting activity are described in Examples 5, 6, 16 and include inhibition of IL-1 binding to IL-1 receptor, inhibition of lymphocyte proliferation or kappa light chain expression, and decrease of IL-1 induced IL-6 expression.
- 15 IL-1R AcP isolated free of other proteins may be obtained from the polynucleotides of this invention which encode IL-1R AcP. For example, IL-1R AcP may be obtained by conventional methods of expressing a polynucleotide provided herein encoding IL-1R AcP, preferably the DNA of [SEQ ID NO: 1] or [SEQ ID NO: 7] in a host cell,
- 20 and isolating the resulting protein. Once the IL-1R AcP is obtained, the protein can be isolated free of other proteins by conventional methods. These methods include but are not limited to purification or antibody affinity columns with the antibodies of this invention, chromatography on ion exchange or gel filtration columns, purification
- 25 by high performance liquid chromatography, and purification with an IL-1 affinity column.
- IL-1R AcP may be stabilized by attaching a polyalkylene glycol polymer by known methods. Polyalkylene glycol includes poly-
- 30 ethylene glycol, and other polyalkylene polymers which may be branched or unbranched. The polymers may be directly linked to the protein, or may be linked by means of linking groups connecting for example the COOH of the polymer to the NH₂ of a lysine on the protein.
- 35 IL-1R AcP of this invention may be used directly in therapy to bind or scavenge IL-1, thereby providing a means for regulating and preventing the inflammatory or immunological activities of IL-1. In

its use to prevent or reverse pathologic responses, soluble IL-1R AcP or antibodies to the IL-1R AcP can be combined with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF receptor, the IL-1 receptor antagonist, soluble IL-1 receptor and the like. In addition, isolated IL-1R AcP of this invention is useful in raising antibodies to IL-1R AcP which are themselves useful in therapy. Raising such antibodies is made feasible because this invention makes available IL-1R AcP in sufficient amounts for antibody production.

10

Thus, this invention is also directed to antibodies to human IL-1R AcP. Murine or rat monoclonal antibodies to human IL-1R AcP are obtained as in Example 15. These antibodies are obtained by immunization with purified or partially purified amounts of human IL-1R AcP, which is obtained after expression of the recombinant full-length or soluble human IL-1R AcP using the DNA's of this invention. The human IL-1R AcP cDNA's were isolated using the murine IL-1R AcP DNA of this invention which was isolated with the unique mAb 4C5 described in Examples 2 and 3. For the murine or rat mAbs to human IL-1R AcP, hybridoma techniques well known in the art may then be used to obtain hybridomas to generate mAbs. Chimeric antibodies and humanized antibodies may be obtained from these rodent antibodies using known methods. (Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663, 1991; WO 90/7861, EP 620276) or by producing heterodimeric bispecific antibodies (Kostelny et al., J. Immunol. 148: 1547, 1992).

Antibodies to human IL-1R AcP of this invention bind specifically to human IL-1R AcP and prevent activation of the IL-1 receptor complex by IL-1. This activity may be determined by assays as described herein. Specifically, biological assays include screens based on the ability of the antibody to inhibit the proliferation of IL-1-responsive cells or the IL-1-induced secretion of prostaglandin E₂ and IL-6. Such assays can be carried out by conventional methods in cell biology. Suitable cells for these assays include splenic B cells, cell lines such as the human B cell line RPMI 1788 (Vandenabeele et al., J. Immunol. Meth. 135: 25, 1990), and human fibroblasts such as the human lung fibroblast line MRC-5 (Chin et al., J. Exp. Med. 165: 70,

1987). Methods for such assays using mouse cells are found in Examples 1, 2, 5, and 6. For example an *in vivo* assay may be used, which measures inhibition of IL-1 induced IL-6 production in mice. These assays may be performed using human cells to effectively 5 screen for the desired activity using the same techniques provided in the Examples. A preferred antibody has a binding affinity to the IL-1 receptor accessory complex of about K_D 0.1 nM to about K_D 10 nM, as determined by conventional methods (Scatchard, Ann. N.Y. Acad. Sci. 51: 660, 1949).

10

The antibodies of this invention may be administered by known methods to relieve conditions caused by the presence of IL-1. In particular, the antibodies of this invention are useful in reducing inflammation. These antibodies to the IL-1R AcP can be administered, 15 for example, for the purpose of suppressing inflammatory or immune responses in a human. A variety of diseases or conditions caused by inflammatory processes (e.g. rheumatoid arthritis, inflammatory bowel disease, and septic shock) or by immune reactions (e.g. Type I diabetes, transplant rejection, psoriasis, and asthma) are associated 20 with elevated levels of IL-1 (Dinarello and Wolff, New Engl. J. Med. 328: 106, 1993). Treatment with antibodies that inhibit IL-1 interaction with the IL-1R AcP may therefore be used to effectively suppress inflammatory or immune responses in the clinical treatment 25 of acute or chronic diseases such as rheumatoid arthritis, inflammatory bowel disease, and Type I diabetes. In addition, antibodies are useful in the treatment of certain cancers, such as acute and chronic myelogenous leukemia (Rambaldi et al., Blood 78: 3248, 1991; Estrov et al., Blood 78: 1476, 1991).

30 Included in this invention are antibodies to murine IL-1R AcP, specifically 4C5, 2B5, 3F1, 4C4, 24C5, 4D4 (see Table 1) and 1D2, 2D6, 2E6, 1F6, 2D4, 2F6, 3F5, and 4A1 (see Table 2). These antibodies are useful to obtain human IL-1R AcP, as described.

35 As noted above, antibodies may be produced naturally by appropriate cells, or may be produced by recombinant expression vectors that modify the antibody proteins, e.g. by humanizing the antibody (Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663, 1991) or

by producing heterodimeric bispecific antibodies (Kostelný et al., J. Immunol. 148: 1547, 1992; WO 90/7861, EP 620276) that can recognize both the accessory protein and the Type I or Type II IL-1R.

5 The dose ranges for the administration of the IL-1R AcP and fragments thereof or of antibodies to the IL-1R AcP or antisense polynucleotides may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for
10 example, blocking the activity of endogenous IL-1 to cells responsive to IL-1. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter-indications, if any,
15 immune tolerance and other such variables, to be adjusted by the individual physician. The IL-1R AcP and fragments thereof or antibodies to this protein or antisense polynucleotides can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally,
20 intramuscularly, or subcutaneously.

This invention includes pharmaceutical compositions comprising the proteins and/or antibodies of this invention in amounts effective to reduce inflammation, and a pharmaceutically acceptable carrier
25 such as the preparations and vehicles described below. Such compositions may include other active compounds if desired. For the proteins, an example of an effective amount is in the range of about 4 to about 32 mg/meter². For antibodies, an example of an effective amount is in the range of about 0.1 to about 15 mg/kg body weight.

30 Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters
35 such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or

fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

The following Examples are provided to further describe the invention and are not intended to limit it in any way.

10

Example 1

Methods

15 Preparation, Screening and Purification of Hybridoma Antibodies

Lewis Rats (Charles River Laboratories) were immunized by the intraperitoneal (i.p) route with detergent solubilized preparations of human IL-1 α (Gubler et al., J. Immunol. 136: 2492, 1986), affinity 20 cross-linked to IL-1R from murine 70Z/3 pre-B cells (ATCC #TIB 158). For the primary immunization, the rats received solubilized IL-1 α /IL-1R complex (0.4 ml) that was prepared and purified from 1×10^{11} 70Z/3 cells (Chizzonite et al., Proc. Natl. Acad. Sci. USA 86: 8029, 1989) and emulsified in Freund's Complete Adjuvant at a 1:2 ratio and 25 injected i.p. (described below). Six weeks later, the rats received solubilized IL-1 α /IL-1R complex (0.3 ml) that was prepared and purified from 2.25×10^{11} cells and emulsified in Freund's Complete Adjuvant at a ratio of 1:2 and injected in each hind foot pad and i.p. Sera were collected from the rats at 2 and 6 weeks after the last 30 immunization and tested for activity that blocked [125 I]-IL-1 β binding to IL-1R on 70Z/3 cells. Four months after the last immunization, one rat was immunized with the following amounts of solubilized IL-1 β /IL-1R complex in preparation for splenocyte isolation: 0.1 ml (prepared and purified from 8×10^{10} cells) 35 emulsified at a 1:4 ratio with Freund's Complete Adjuvant and injected in each hind foot pad and subcutaneous (s.c.) in each hind limb, and 0.9 ml (prepared and purified from 7.4×10^{11} 70Z/3 cells) injected intravenous (i.v.) and i.p. Two days later, the rat was immunized with solubilized IL-1 α /IL-1R complex (0.5 ml; prepared

and purified from 2×10^{11} 70Z/3 cells) mixed with phosphate buffered saline (PBS), pH 7.4 (0.5 ml) and injected s.c. in each hind limb. Two days after this last immunization, spleen cells were isolated from the rat and fused with SP2/0 cells (ATCC CRL 1581) at a ratio of 5 1:1 (spleen cells:SP2/0 cells) with 35% polyethylene glycol (PEG 4000, E. Merck) according to a published procedure (Fazekas et al., J. Immunol. Meth. 35: 1, 1980). The fused cells were plated at a density of 3×10^5 cells/well/ml in 48 well plates in IMDM supplemented with 15% FBS, glutamine (2 mM), beta-mercaptoethanol (0.1 mM), 10 gentamicin (50 µg/ml), HEPES (10 mM), 5% ORIGIN hybridoma cloning factor (IGEN, Inc.), 5% P388D1 supernatant (Nordon et al. J. Immunol. 139: 813, 1987) and 100 Units/ml recombinant human IL-6 (Genzyme).

15 Hybridoma supernatants were screened for inhibitory and non-inhibitory antibodies specific for an IL-1R AcP and the Type II IL-1R in four assays: 1) for inhibitory antibodies: inhibition of [¹²⁵I]-IL-1β binding to 70Z/3 and EL-4 thymoma cells (described below), 2) for non-inhibitory antibodies: immunoprecipitation of solubilized complex 20 of [¹²⁵I]-IL-1β crosslinked to Type II IL-1R, 3) for inhibitory antibodies specific for IL-1R AcP or Type II IL-1R: inhibition of [¹²⁵I]-IL-1β and [¹²⁵I]-IL-1α binding to cells expressing recombinant Type I and Type II IL-1Rs, and 4) to eliminate any 25 antibodies specific for IL-1: immunoprecipitation of [¹²⁵I]-IL-1α and [¹²⁵I]-IL-1β. Hybridoma cell lines secreting antibodies specific for Type II IL-1R and the IL-1R AcP were cloned by limiting dilution. Antibodies were purified from large scale hybridoma cultures or ascites fluids by affinity chromatography on protein G bound to Sepharose 4B fast flow according to the manufacturer's protocol 30 (Pharmacia).

Cultured Cells and Biological Assays

Mouse EL-4.IL-2 thymoma cells (TIB 181) and D10.G4.1 (TIB 35 224) cells were maintained as previously described (Kilian et al., J. Immunol. 136: 1, 1986). Mouse 3T3L1 (CL 173) and 70Z/3 pre-B (TIB 158) cells were maintained in IMDM containing 5% fetal bovine serum

in 600 cm² dishes. The above cells were obtained from the American Type Culture Collection and the ATCC numbers are in parenthesis.

The biological activity of unlabeled IL-1 and [¹²⁵I]-IL-1
5 proteins were evaluated in the murine D10 proliferation assay (Kaye et al., J. Exp. Med. 158: 836, 1983).

Labeling of IL-1 and Purified Monoclonal Antibodies with ¹²⁵I

10 Recombinant murine IL-1 α , human IL-1 α and human IL-1 β were purified as previously described (Kilian et al., J. Immunol. 136: 1, 1986; Gubler et al., J. Immunol. 136: 2492, 1986) except that murine IL-1 α was prepared in 25 mM Tris-HCl, 0.4 M NaCl. Protein determinations were performed by BCA protein assay (Pierce
15 Chemical Co., Rockford, IL). Human IL-1 α human IL-1 β , murine IL-1 α , murine IL-1 β and purified IgG were labeled with ¹²⁵I by a modification of the Iodogen method (Pierce Chemical Co.). Iodogen was dissolved in chloroform and 0.05 mg dried in a 12 x 15 mm borosilicate glass tube. For radiolabeling, 1.0 mCi Na[¹²⁵I]
20 (Amersham, Chicago, IL) was added to an Iodogen-coated tube containing 0.05 ml of Tris-iodination buffer (25 mM Tris-HCl pH 7.5, 0.4 M NaCl, 1 mM EDTA) and incubated for 4 min at room temperature. The activated ¹²⁵I solution was transferred to a tube containing 0.05 to 0.1 ml IL-1 (5-13 μ g) or IgG (100 μ g) in Tris-
25 iodination buffer and the reaction was incubated for 5-8 min at room temperature. At the end of the incubation, 0.05 ml of Iodogen stop buffer (10 mg/ml tyrosine, 10% glycerol in Dulbecco's PBS, pH 7.4) was added and reacted for 3 min. The mixture was then diluted with 1.0 ml Tris-iodination buffer, and applied to a Bio-Gel P10DG desalting
30 column (BioRad Laboratories) for chromatography. The column was eluted with Tris-iodination buffer, and fractions (1 ml) containing the peak amounts of labeled protein were combined and diluted to 1 x 10⁸ cpm/ml with 1% BSA in Tris-iodination buffer. The TCA precipitable radioactivity (10% TCA final concentration) was typically
35 in excess of 95% of the total radioactivity. The radiospecific activity was typically 2000 to 3500 cpm/fmol for purified antibodies and 3500 to 4500 cpm/fmole for IL-1.

Mouse IL-1 Receptor Binding Assays

Binding of radiolabeled IL-1 to mouse cells grown in suspension culture was measured by a previously described method (Kilian et al., 5 J. Immunol. 136: 1, 1986). Briefly, cells were washed once in binding buffer (RPMI-1640, 5% FBS, 25 mM HEPES, pH 7.4), resuspended in binding buffer to a cell density of 1.5×10^7 cells/ml and incubated (1.5 x 10^6 cells) with various concentrations of [125 I]-IL-1 (5-1000 pM) at 4°C for 3-4 hrs. Cell bound radioactivity was separated from 10 free [125 I]-IL-1 by centrifugation of the assay mixture through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 : A.H. Thomas, and Silicone Oil AR 200 : Gallard-Schlessinger) at 4°C for 90 sec at 10,000 x g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non- 15 specific binding was determined by inclusion of 50 nM unlabeled IL-1 in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed by using the non-linear regression programs EBDA, LIGAND and Kinetic (Munson and Rodbard, Anal. Biochem 107: 220, 1980) as adapted for the IBM personal 20 computer by McPherson (McPherson, J. Pharmacol. Methods 14: 213, 1985) from Elsevier-BIOSOFT.

The binding of radioiodinated IL-1 proteins to adherent cells was performed by incubating cells and ligands in a 24 or 12 well plate 25 at 4°C on a rocker platform for 4 hrs in binding buffer (24). Monolayers were then rinsed 3 times with binding buffer at 4°C, solubilized with 0.5 ml 1% SDS and the released radioactivity counted in a gamma counter. Non-specific binding was determined in the presence of 50 nM unlabeled IL-1. Analysis of the binding data was 30 performed as described above.

Equilibrium Binding of [125 I]-labeled Monoclonal Antibodies to Murine Cells

35 Murine cells were washed once in binding buffer (RPMI 1640, 5% FBS, 25 mM Hepes, pH 7.4) and resuspended in binding buffer to a cell density of 1.5×10^7 cells/ml. Cells (1.5 x 10^6) were incubated with various concentrations of [125 I]-specific IgG (.005 to 2 nM) at

room temperature for 1.5-2 hrs. Cell bound radioactivity was separated from free [¹²⁵I]-labeled antibody by centrifugation of the assay mixture through 0.1 ml silicone oil at 4°C for 90 seconds at 10,000 x g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 100 nM unlabeled antibody in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed as described above for IL-1 binding to cells.

10

Antibody Mediated Inhibition of [¹²⁵I]-IL-1 Binding to Murine Cells Bearing Type I or Type II IL-1 Receptors

The ability of hybridoma supernatant solutions, purified IgG, or antisera to inhibit the binding of [¹²⁵I]-IL-1 proteins to murine cells bearing IL-1 receptor was measured as follows: serial dilutions of culture supernatants, purified IgG or antisera were mixed with cells (1-1.5 x 10⁶ cells) in binding buffer (RPMI-1640, 5% FBS, 25 mM Hepes, pH 7.4) and incubated on an orbital shaker for 1 hour at room temperature. [¹²⁵I]-IL-1 (1 x 10⁵ cpm; 25 pM) was added to each tube and incubated for 3-4 hours at 4°C. Non-specific binding was determined by inclusion of 50 nM unlabeled IL-1 in the assay. Incubations were carried out in duplicate or triplicate. Cell bound radioactivity was separated from free [¹²⁵I]-IL-1 by centrifugation of the assay through 0.1 ml of an oil mixture as described above. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter.

Affinity Cross-linking and Purification of Solubilized [¹²⁵I]-IL-1 α /IL-1R Complexes

Affinity cross-linking of radioiodinated IL-1 proteins to cells was performed as described (Riske et al., J. Biol. Chem. 266: 11245, 1991) with minor modifications. Briefly, cells (1.5 x 10⁷ cells/ml) were incubated with radiolabeled IL-1 (60-300 fmoles/ml) in the presence or absence of 50 nM unlabeled IL-1 for 4 hrs at 4°C in binding buffer. The cells were then washed with ice cold PBS, pH 8.3 (25 mM sodium phosphate, pH 8.3, 0.15 M NaCl, 1 mM MgCl₂),

- resuspended at a concentration of 5×10^6 cells/ml in PBS, pH 8.3. Disuccinimidyl suberate (DSS) or bis(sulfosuccinimidyl)suberate (BS3) (Pierce Chemical Co.) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30-60 min at 5 4°C with constant agitation. The cells were washed with ice cold 25 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 5 mM EDTA and solubilized at 0.5-1 $\times 10^8$ cells/ml in solubilization buffer (50 mM sodium phosphate, pH 7.5, containing either 8 mM CHAPS or 1% Triton X-100, 0.25 M NaCl, 5 mM EDTA, 40 $\mu\text{g}/\text{ml}$ phenylmethylsulfonyl fluoride, and 0.05% NaN₃) 10 for 1 hr at 4°C . The detergent extract was centrifuged at 120,000 x g for 1 hr at 4°C to remove nuclei and other debris. The extracts were directly analyzed by SDS-PAGE on 8% pre-cast gels (NOVEX) followed by autoradiography. Alternatively, the extracts were immunoprecipitated with antibody bound to Gamma-Bind G Plus (Pharmacia). 15 The precipitated proteins were released by treatment with Laemmli sample buffer (Laemmli, Nature 227: 680, 1970), separated by SDS-PAGE and analyzed by autoradiography.

- Preparation of the solubilized crosslinked complex of IL-1 α /IL-1R that was used as the immunogen was performed as described above with minor modifications. Briefly, 70Z/3 cells (0.5-1.0 $\times 10^8$ cells/ml) were incubated with IL-1 α (0.5 to 1.0 nM) for 4 hrs at 4°C in binding assay buffer. The cells were then washed with ice cold PBS, pH 8.3, resuspended at a concentration of 5×10^7 cells/ml in PBS, pH 25 8.3 and bis(sulfosuccinimidyl)suberate (BS3) (Pierce Chemical Co.) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30-60 min at 4°C with constant agitation. The quenching of the affinity crosslinking procedure and the detergent solubilization of the cells was as described above.

- 30 For purification of the solubilized IL-1 α /IL-1R complex that was used as the immunogen, the detergent extract of 70Z/3 cells was applied to an affinity column (10 ml) of goat anti-human IL-1 α immobilized on crosslinked beaded agarose (Affi-Gel 10, BioRad 35 Laboratories). The goat anti-human IL-1 α affinity column was prepared according to the manufacturer's instructions at a density of 1 mg of IgG/ml of packed gel. After application of the detergent extract, the column was washed with 10 column volumes of

solubilization buffer without Chaps or Triton X-100 or until the absorbance at 280nM was at baseline. The column was then eluted with 3 M potassium thiocyanate, 25 mM sodium phosphate, pH 7.5, 5 mM EDTA, 40 µg/ml phenylmethylsulfonyl fluoride, and 0.05% NaN₃.

- 5 The proteins eluted from the affinity column were concentrated 10 to 100 fold and used for immunization.

Immunoblot Analysis of Proteins Solubilized from Murine Cells

10 Murine 70Z/3 and EL-4 cells were washed 3 times with ice-cold PBS and solubilized at 0.5 - 1 x 10⁸ cells/ml in solubilization buffer that contained either 8 mM CHAPS or 1% Triton X-100 and 1 mg/ml BSA for 1 hr at 4°C. The extracts were centrifuged at 120,000 x g for 45 min at 4°C to remove nuclei and other debris. The extracts were
15 incubated with either 4C5 (anti-IL-1R AcP obtained as described in Example 2), 12A6 (anti-Type I IL-1R obtained as described in Chizzonite et al., Proc. Natl. Acad. Sci. USA 86:8029, 1989) or control antibody bound to protein-G immobilized on crosslinked agarose (Gamma Bind G Plus, Pharmacia). The precipitated proteins were
20 released by treatment with 0.1 M glycine pH 2.3, neutralized with 3M Tris, mixed with 1/5 volume of 5X Laemmli sample buffer, and separated by SDS/PAGE on 8% pre-cast acrylamide gels (NOVEX). The separated proteins were transferred to nitrocellulose membrane (0.2 µM) for 16 hours at 100 volts in 10 mM Tris-HCl pH 8.3, 76.8 mM
25 glycine, 20% methanol and 0.01% SDS. The nitrocellulose membrane was blocked with BLOTTTO (50% w/v nonfat dry milk in PBS + .05% Tween 20) and duplicate blots were probed with [¹²⁵I]-4C5 IgG (1 x 10⁶ cpm/ml in 8mM CHAPS, PBS, 0.25 M NaCl, 10% BSA and 5 mM EDTA) with and without unlabeled 4C5 IgG (67nM).

30 Expression of Murine Recombinant Type I and Type II IL-1 Receptors and IL-1R AcP in COS Cells and Determination of [¹²⁵I]-labeled 4C5, 35F5 and IL-1 Binding

35 COS cells (4-5 x 10⁷) were transfected by electroporation with 25 µg of plasmid DNA expressing recombinant murine IL-1R proteins or IL-1R AcP in a BioRad Gene Pulser (250 µF, 250 volts) according to the manufacturer's protocol. The cells were plated in a 600 cm²

culture plate, harvested after 72 hours by treatment with No-Zyme (JRH Biologics) and scraping, washed and resuspended in binding buffer. Transfected cells ($4-8 \times 10^4$) were incubated with increasing concentrations of [125 I]-labeled 4C5, 35F5 or IL-1 proteins at 4°C for 5 3 hrs. Cell bound radioactivity was separated from free [125 I]-labeled antibody or IL-1 as described above.

Kappa Light Chain Expression by 70Z/3 Cells in Response to IL-1:
Inhibition by Monoclonal Antibodies 35F5, 4E2 and 4C5

10

70Z/3 cells (1×10^5 /ml in RPMI 1640, supplemented with 10% FBS, β -mercaptoethanol and gentamicin) were incubated with and without 100 U/ml (0.19 nM) of human recombinant IL-1 α or IL-1 β for 24 hrs or 48 hrs. The cells were preincubated for one hour before 15 the addition of IL-1 with 30 μ g/ml of the indicated antibodies in a total volume of 0.5 ml. An additional 0.5 ml of medium containing the IL-1 or medium alone was added to the wells for a final concentration of 15 μ g/ml (100 nM) antibodies. The cells were washed once after culture and stained with either a control rat antibody conjugated with 20 FITC or rat anti-mouse kappa light chain antibody conjugated with FITC (Tago, Burlingame, Ca). The cells were then analyzed for kappa light chain expression on a FACScan flow cytometer (Becton-Dickinson).

25 Proliferation of Murine Splenic B cells in Response to IL-1: Inhibition by Monoclonal Antibodies 35F5, 4C5 and 4E2.

Splenic B cells were purified by treating splenocytes isolated from C57BL/6 mice with anti-Thy1.2 antibody and rabbit 30 complement, followed by two sequential passages through a Sephadex G10 (Pharmacia) columns. B cells (5×10^5 cells) were treated with goat anti-mouse IgM (1 μ g/ml) (ZYMED) and dibutyryl cAMP (10^{-3} M) in a final volume of 200 μ l of RPMI 1640 media supplemented with 10% FBS, β -mercaptoethanol and gentamicin. Splenic B cells were 35 treated with and without IL-1 (100 U/ml) and with and without antibodies 35F5, 4C5 and 4E2. The cells were incubated for two days in the presence of the various reagents and then pulsed with 0.5 μ Ci tritiated thymidine, incubated for an additional 6 hrs, and harvested.

Proliferation of Murine D10.G4.1 Cells in Response to IL-1: Inhibition by Monoclonal Antibodies 4C5 and 35F5 and Human IL-1ra

5 D10.G4.1 helper T cells were maintained as described (Kaye et al., J. Exp. Med. 158: 836, 1983; McIntyre et al., J. Exp. Med. 173: 931, 1991) and stimulated with IL-1 as previously described (McIntyre et al., J. Exp. Med. 173: 931, 1991). Cells (1×10^5 in 200 μ l) were incubated with 0.2 pM IL-1 in RPMI 1640 containing 5% FBS,
10 β -mercaptoethanol (5×10^{-5} M), gentamicin (8 μ g/ml), 2 mM L-glutamine, 2.5 μ g/ml concanavalin A and the indicated concentrations of antibodies or human IL-1 receptor antagonist (IL-1ra). The cultures were incubated for two days, pulsed with 0.5 μ Ci tritiated thymidine and harvested 16 hrs later.

15 *In Vivo* Induction of Serum IL-6 by IL-1: Inhibition by Monoclonal Antibodies 35F5 and 4C5

The induction of serum IL-6 by IL-1 was performed as
20 previously described (McIntyre et al., J. Exp. Med. 173: 931, 1991). Briefly, C57BL/6 mice were pretreated (i.p) with 250 μ g of antibody at 4 hrs and 10 min before administration of IL-1 α or IL-1 β (0.3 μ g/mouse, s.c.). Sera were collected from the mice 2 hrs after administration of IL-1 and analyzed for IL-6 concentration by a
25 modification of the B9 hybridoma cell bioassay as described (Aarden et al., Eur. J. Immunol. 17: 1411, 1987).

The rat anti-mouse IL-1 accessory protein monoclonal antibody 4C5 was prepared, characterized and generated as follows:

30

Example 2

Preparation, Characterization and Identification of Monoclonal Antibodies Specific for IL-1R AcP and Type II IL-1R

35

In the course of preparing antibodies to the Type II IL-1 receptor, antibodies to an unexpected, novel component of the IL-1 receptor complex were detected. Since murine 70Z/3 cells express

almost exclusively the Type II IL-1R, immunization of rats with the purified crosslinked IL-1 α /IL-1R complex solubilized from these cells was the initial strategy pursued to develop monoclonal anti-Type II IL-1R antibodies. Rats immunized with this solubilized IL-1 α /IL-1R complex developed serum antibodies that blocked [125 I]-IL-1 β binding to 70Z/3, indicating the presence of blocking antibodies specific for the Type II IL-1R. The serum samples also contained antibodies that immuno-precipitated the [125 I]-IL-1 β /IL-1R complex solubilized from 70Z/3 cells, indicating the presence of non-blocking anti-Type II IL-1R antibodies. [125 I]-IL-1 β was used for the IL-1R binding and immunoprecipitation assays to eliminate identification of antibodies specific for IL-1 α instead of the Type II receptor.

Hybridomas resulting from the fusion of splenocytes isolated from the immunized rat were screened for antibodies that blocked IL-1 β binding to both 70Z/3 (Type II receptor bearing) and EL-4 (Type I receptor bearing) cells. Antibodies that block binding only to 70Z/3 cells were identified and eliminated from further analysis because they are antibodies to Type II IL-1R, and antibodies that blocked binding only to EL-4 cells were identified and eliminated from further analysis because they are antibodies to Type I IL-1R. Antibodies that blocked IL-1 binding to both cell types are specific for the IL-1R AcP.

From the initial fusion, seven antibodies were identified that blocked IL-1 β binding to 70Z/3 cells (Table 1). Six of these antibodies (2B5, 4C5, 3F1, 4C4, 24C5, and 4D4) blocked IL-1 β binding to both 70Z/3 and EL-4 cells. These antibodies did not block IL-1 β binding to CHO cells expressing murine recombinant Type I IL-1R, and were therefore specific for an IL-1R AcP. One antibody, 4E2, only blocked IL-1 β binding to 70Z/3 cells, indicating that it was specific for the Type II IL-1R.

The initial fusion was also screened for non-blocking antibodies that were specific for either the IL-1R AcP or the Type II IL-1R. Eight antibodies (1D2, 2D6, 2E6, 1F6, 2D4, 2F6, 3F5 and 4A1) immuno-precipitated the IL-1 β /IL-1R complex solubilized from 70Z/3 cells (Table 2). These antibodies also immunoprecipitated the IL-1 β /IL-1R

complexes solubilized from two other Type II IL-1R bearing murine cell lines, AMJ2C11 and P388D1. Seven of these antibodies also immunoprecipitated the IL-1B/IL-1R complex solubilized from EL-4 cells, demonstrating that they recognized an IL-1R AcP. One antibody, 5 1F6, did not bind to the IL-1B/IL-1R complex solubilized from EL-4

Table 1 Identification of Inhibitory Anti-IL-1R AcP Antibodies

1. Inhibition of [¹²⁵I]-IL-1 β binding to cell lines, 7OZ/3, AMJ2C11, P388D1, EL-4 and CHO (Mu Type I IL-1R) by antibodies was described in Example 1.
2. Immunoprecipitation of [¹²⁵I]-labelled recombinant IL-1 proteins was as described in Example 1.
3. rHuIL-1 α = human recombinant IL-1 α .
rHuIL-1 β = human recombinant IL-1 β .
rMuIL-1 α = murine recombinant IL-1 α .
4. ++ and +; antibody blocks [¹²⁵I]-IL-1 β Binding.
5. -; antibody was negative in the assay.

Table 2
Identification of Non-Inhibitory Anti-IL-1R AcP Antibodies

Ligand	Monoclonal Antibody						4A1
	1D2	2D6	2E6	1F6	2D4	2F6	
Immunoprecipitation¹							
rHuIL-1 α	-	-	-	-	-	-	-
rHuIL-1 β	-	-	-	-	-	-	-
rMuIL-1 α	-	-	-	-	-	-	-
Immunoprecipitation of Crosslinked Complexes²							
7OZ ³ (Type II IL-1R)	+ +	+ +	+ +	+ +	+ +	+ +	-
AMJ2C11 (Type II IL-1R)	+ +	+ +	+ +	+ +	+ +	+ +	-
P388D1 (Type II IL-1R)	+ +	+ +	+ +	+ +	+ +	+ +	-
EL-4 (Type I IL-1R)	+ +	+ +	+ +	+ +	+ +	+ +	-
sMu Type I IL-1R (bv) ³	-	-	-	-	-	-	-
Direct Immunoprecipitation Assay⁴							
[¹²⁵ I-sMu IL-1R(bv)]	-	-	-	-	-	-	-
[¹²⁵ I-sMu IL-1R(Cos)]	-	-	-	-	-	-	-

1. As described in Table 1.
2. Immunoprecipitation of the complex of [¹²⁵I]-IL-1 β /IL-1R solubilized from the indicated cell lines.
3. Immunoprecipitation of the complex of [¹²⁵I]-IL-1 β affinity crosslinked to the soluble murine Type I IL-1R expressed in a baculovirus system.
4. Immunoprecipitation of [¹²⁵I]-labelled soluble Type I IL-1R expressed in either baculovirus [¹²⁵I-sMuIL-1R (bv)] or Cos cell [¹²⁵I-sMsR(Cos)] expression systems.

cells, indicating it was a non-blocking Type II IL-1R antibody. To confirm that these antibodies did not bind to the Type I IL-1R, they were tested in immunoprecipitation assays with murine soluble Type I IL-1R (Table 2). None of these antibodies immunoprecipitated the complex of [¹²⁵I]-IL-1 β crosslinked to recombinant soluble Type I IL-1R ([¹²⁵I]-sMsR[bv]). They also did not immunoprecipitate [¹²⁵I]-labeled soluble Type I receptor produced either in a baculovirus/insect cell expression system or in a COS cell expression system (Table 2).

10

Since the rats were immunized with the solubilized IL-1 α /IL-1R complex, antibodies in the rat serum were also detected that recognized IL-1 α . Each monoclonal antibody was tested in immunoprecipitation assays with [¹²⁵I]-labeled murine and human IL-1 proteins to confirm that they did not bind to IL-1. All 15 antibodies (Tables 1 and 2) were negative in these assays.

15

20 Characterization of Murine IL-1Rs and IL-1R AcP by Reactivity with Anti-Type I (35F5), Type II (4E2) and Accessory Protein (4C5) Monoclonal Antibodies

25

Following the initial identification and characterization of the antibodies described above, 4C5, a putative blocking IL-1R AcP (IL-1R AcP) antibody, and 4E2, a blocking Type II IL-1R antibody, were chosen as probes for the further study of the IL-1R AcP. A previously identified and characterized anti-Type I IL-1R antibody, 35F5, was also included in this study (Chizzonite et al., Proc. Natl. Acad. Sci. USA 86: 8029, 1989; McIntyre et al., J. Exp. Med. 173: 931, 1991).

30

These three antibodies were used to identify the presence of Type I and Type II IL-1R's and IL-1R AcP on various murine cells. Equilibrium binding assays with [¹²⁵I]-labeled mAb 4C5 demonstrated the presence of IL-1R AcP on murine cells bearing predominately Type I (EL-4 cells) or Type II (70Z/3 cells) receptors (Figures 1 and 2). Other cells bearing predominately Type I (3T3L1 cells) or Type II (P388D1 cells) receptors also expressed IL-1R AcP

(Table 3). Cells (S49.1) that do not express either Type I or Type II IL-1R AcP did not express IL-1R AcP, indicating a link between expression of IL-1R and IL-1R AcP. During its initial characterization, mAb 4C5 blocked [¹²⁵I]-human IL-1 β binding to 5 both EL-4 and 70Z/3 cells. Further studies established

Table 3
Equilibrium Binding of Radiolabelled IL-1, 4C5 and 4E2
to Murine Cells Expressing Predominantly Type I or Type II IL-1Rs

Ligand	CELL LINE		S49.1 ⁸		3T3L ₁ ⁷		70Z/3 ⁴		P388D1 ⁴		Cos 11 ⁴	
	EL-4 ⁷	K _D ²	S/C ³	K _D	S/C	K _D	S/C	K _D	S/C	K _D	S/C	
[¹²⁵ I]-IL-1 _I												
rMuIL-1 α	.05	1200	NSB ⁵	.008	1640	.2	1500	.19	380	.21	1950	
rHuIL-1 α	.05	1200								.33	1200	
rHuIL-1 β	.1	1200										
[¹²⁵ I]-4C5	1.2	2800	NSB	.93	19200	1.4	3000	.77	1870	NSB		
[¹²⁵ I]-4E2	NSB		NSB	.61	14200			1.2	1900	ND	ND	

1. Abbreviations of IL-1 proteins as in Table 1.
2. K_D = equilibrium dissociation constant (nM).
3. S/C = binding sites per cell.
4. Cells expressing murine natural or recombinant Type II IL-1R.
5. NSB = no specific binding of the radiolabelled ligand.
6. ND = not determined.
7. Cells expressing murine natural Type I IL-1R.
8. Cells not expressing either murine Type I or Type II IL-1Rs.

that mAb 4C5 also inhibited the binding of radiolabeled human IL-1 α (Fig. 3), murine IL-1 α and IL-1 β to 70Z/3 cells (Table 4). Similar to its inhibition of [125 I]-human IL-1 β binding to EL-4 cells, 4C5 also blocked [125 I]-murine IL-1 β binding to these cells (Table 4).

5 However, 4C5 did not block either radiolabeled human IL-1 α (Fig. 4) or murine IL-1 α (Table 4) binding to EL-4 cells. Moreover, 4C5 did not block the binding of [125 I]-labeled IL-1 proteins to CHO or COS cells expressing murine recombinant Type I or Type II receptors. The anti-Type I receptor antibody, 35F5, and the anti-Type II receptor 10 antibody, 4E2, inhibited both IL-1 α and IL-1 β binding to their respective IL-1 receptors, regardless of whether the receptors were the natural or recombinant forms (Table 4). The IC₅₀s for 4C5-mediated inhibition of IL-1 binding to EL-4 and 70Z/3 cells were at least 1000-fold lower than IC₅₀s for inhibition of binding to cells 15 expressing recombinant Type I or Type II receptors (Table 5). These IC₅₀ data suggested two conclusions: 1) mAb 4C5 did not crossreact to any significant extent with Type I or Type II IL-1R's, and 2) the difference in the ability of 4C5 to block IL-1 β , but not IL-1 α , binding to natural IL-1R's was unrelated to the affinity of the antibody.

20

Example 4

Determination of the Size of the IL-1R AcP Recognized by Monoclonal Antibody 4C5

25

The approximate molecular size of the cell surface protein recognized by mAb 4C5 on EL-4 cells was determined by affinity chromatography and immunoblotting to be approximately 90 kDa (Fig. 5). Detergent extracts prepared from EL-4 cells were purified on a lentil lectin affinity matrix followed by affinity chromatography on either an anti-Type I receptor antibody (7E6), murine IL-1 α (Ma) or 4C5 affinity gel. 30 The proteins eluted from each affinity column were treated with Laemmli sample buffer, separated by SDS-PAGE on 8% gels and transferred to nitrocellulose membrane. The proteins immobilized on 35 the nitrocellulose were probed with [125 I]-4C5 and the immuno-reactive bands identified

Table 4
Inhibition of Binding of IL-1 Proteins to Different Subtypes and Forms of the Murine IL-1 Receptor by Anti-Receptor Antibodies

IL-1s	Type I				Type II	
	Natural	Recombinant	Natural	Recombinant		
	EL-4 ⁴	CHO ⁶	T _O Z/3 ⁵	cOS ⁸		
Inhibition by 35F5 (anti-Type I)						
Mu IL-1 α	+	+	-	-	-	-
Mu IL-1 β	+	+	ND	-	ND	-
Hu IL-1 α	+	+	+	-	ND	-
Hu IL-1 β	+	+	+	-	ND	-
Hu IL-ra	50%	ND	+	-	ND	-
Inhibition by 4C5 (anti-Accessory Protein)						
Mu IL-1 α	-	-	-	-	-	-
Mu IL-1 β	+	-	ND	+	ND	-
Hu IL-1 α	-	-	ND	+	-	-
Hu IL-1 β	+	-	ND	+	-	-
Hu IL-1ra	-	ND	ND	ND	ND	-
Inhibition by 4E2 (anti-Type II)						
Mu IL-1 α	-	ND	ND	+	+	-
Mu IL-1 β	-	ND	ND	+	ND	-
Hu IL-1 α	-	ND	ND	+	+	-
Hu IL-1 β	-	ND	ND	+	+	-

1. + and -; Antibody blocks 100% and less than 10%, respectively, of IL-1 binding at 0.1 mg/ml.
2. Mu IL-1 α and Mu IL-1 β = murine IL-1 α and IL-1 β , respectively.
3. Hu IL-1 α , Hu IL-1 β , and Hu IL-1ra = human IL-1 α , IL-1 β , and IL-1ra, respectively.
4. Murine EL-4 cells express approximately 2000 Type I IL-1Rs/cell and an undetectable number of the Type II IL-1R.
5. Murine 7OZ/3 cells express approximately 2000 Type II IL-1Rs/cell and undetectable numbers of Type I IL-1R.
6. Recombinant full length Type I IL-1R expressed in CHO cells.
7. Recombinant Type I IL-1R extracellular domain expressed in a baculovirus system.
8. Recombinant Type II IL-1R expressed in COS cells.

Inhibition of IL-1 Binding to Different IL-1 Receptors by Anti-Receptor Antibodies

Antibody	IC ₅₀ (µg/ml)	Type I				Type II			
		Natural ¹	Recombinant ²	Natural ¹	Recombinant ²	7OZ/3	HuIL-1α	HuIL-1β	HuIL-1α
EL-4									
HuIL-1α ³	HuIL-1β ⁴	HuIL-1α	HuIL-1β	HuIL-1α	HuIL-1β	>100	>100	>100	ND
3SF5 (anti-Type I)	.0001	.0015	<.1	<.1	<.1				ND
4E2 (anti-Type II)	>100	>100	>100	>100	>100	.1	.25	2	ND
4C5 (anti-accessory protein)	>100	.13	>100	>100	>100	.32	.34	>100	>100

1. Source of natural Type I (EL-4 cells) and Type II (7OZ/3 cells) IL-1Rs used in the inhibition assays.

2. Recombinant Type I or Type II IL-1Rs were expressed in either CHO or COS cells.

3. [¹²⁵I]-Hu IL-1α as ligand in the assay.

4. [¹²⁵I]-Hu IL-1β as ligand in the assay.

by autoradiography. A major protein of ~90 kDa and a minor protein of 55 kDa were immunoreactive with radiolabeled 4C5. These two proteins were also identified on the immunoblot if the EL-4 extract was directly purified on a 4C5 affinity matrix. These data indicated
5 that the apparent molecular weight of the natural, glycosylated IL-1R AcP is ~90 kDa and that proteolytic processing may reduce its size to ~55 kDa.

Example 5

10

Neutralization of IL-1 β Biologic Activity by Monoclonal Antibody 4C5

The ability of mAb 4C5 to neutralize IL-1 β biologic activity in a dose-dependent manner was demonstrated in three biologic assays: 1)
15 IL-1 induced proliferation of murine splenic B cells, 2) IL-1 induced proliferation of D10.G4.1 helper T cells, and 3) IL-1 induced kappa light chain expression in 70Z/3 cells. MAb 4C5 demonstrated a dose-dependent inhibition of IL-1 β , but not IL-1 α , induced proliferation of the splenic B cells (Fig. 6). In contrast to mAb 4C5, the anti-Type I
20 receptor antibody 35F5 blocked both IL-1 α and IL-1 β induced proliferation of B cells. The anti-Type II IL-1R antibody 4E2 did not inhibit proliferation induced by either IL-1 α or IL-1 β . In a similar fashion, mAb 4C5 inhibited IL-1 α , but not IL-1 β , induced proliferation of D10.G4.1 T cells (Fig. 7). Both mAb 35F5 and human IL-1ra blocked
25 IL-1 α and IL-1 β induced proliferation of the D10.G4.1 cells. MAb 4C5 also blocked IL-1 β , but not IL-1 α , induced expression of kappa light chain on 70Z/3 cells (Fig. 8). Antibody 35F5 blocked both IL-1 α and IL-1 β induced effects in this assay, whereas mAb 4E2, which recognizes the Type II IL-1R, was inactive. For these assays,
30 neutralization of IL-1 activity by the antibodies or by IL-1ra is detected as a dose-dependent decrease in the biological response. The block in response may be 100% inhibition (i.e. equal to no IL-1 added) or to a lower level depending on the potency of the antibody.

Example 6**Inhibition of IL-1 β Biologic Activity *In Vivo* by Monoclonal Antibody 4C5**

5

Mice administered IL-1 show a rapid and dramatic increase in the concentration of IL-6 in their serum. The magnitude of the increase in serum IL-6 is dependent on the IL-1 dose and can be blocked by factors that interfere with IL-1 binding to Type I IL-1R.

- 10 When tested in this IL-1 biological model, 4C5 blocked by approximately 90% the IL-1 β , but not IL-1 α , induced increase in serum IL-6 (Fig. 9). The anti-Type I IL-1R antibody 35F5 blocked both IL-1 α and IL-1 β induced increase in serum IL-6. A control mAb X-7B2 had no inhibitory effect.

15

Example 7**Expression cloning of Mouse (Murine) IL-1R AcP using Mab 4C5**

20 Extraction of RNA

- 3T3-L1 cells were harvested and total RNA was extracted using guanidinium isothiocyanate/phenol as described (P. Chomczynski and N. Sacchi, Anal. Biochem. 162:156, 1987). Poly A $^+$ RNA was isolated from total RNA by one batch adsorption to oligo dT latex beads as described (K. Kurabayashi *et al.*, Nucl. Acids Res. Symposium Series 19: 61, 1988). The mass yield of poly A $^+$ RNA from this purification was approximately 6%. The integrity of the RNA preparations was analyzed by fractionating in 1.0% agarose gels under denaturing conditions in the presence of 2.2M formaldehyde (Molecular Cloning, 30 A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, 1989).

3T3-L1 cDNA library construction

- From the above poly A $^+$ RNA, a cDNA library was established in the 35 mammalian expression vector pEF-BOS (Mizushima and Nagata, Nucl. Acids Res. 18: 5322, 1990). 10 μ g of poly A $^+$ RNA were reverse transcribed using RNaseH $^-$ reverse transcriptase (GIBCO BRL Life Technologies Inc., Gaithersburg, MD). The resulting mRNA-cDNA

hybrids were converted into blunt ended doublestranded cDNAs by established procedures (Gubler and Chua, in: Essential Molecular Biology, Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991). *Bst*XI linkers (Aruffo and Seed, Proc. Natl. Acad. Sci (USA) 84:8573, 5 1987) were ligated to the resulting cDNAs and molecules >1000 base pairs (bp) were selected by passage over a Sephadryl SF500 column. The Sephadryl SF500 column (0.8 x 29 cm) was packed by gravity in 10mM Tris-HCl pH 7.8/1mM EDTA/100mM NaAcetate. *Bst*XI linker-treated cDNA was applied to the column and 0.5 ml fractions were 10 collected. A small aliquot of each fraction was fractionated in a 1.0% agarose gel. The gel was dried down by vacuum and the size distribution of the radioactive cDNA was visualized by exposure of the gel to X-ray film. Fractions containing cDNA molecules >1000 bp were selected and pooled. The cDNA was concentrated by ethanol 15 precipitation and ligated to the cloning vector. The cloning vector was the plasmid pEF-BOS that had been digested with *Bst*XI restriction enzyme and purified over two consecutive agarose gels. 375 ng of plasmid DNA were ligated to 18.75 ng of size selected cDNA from above in 150 µl of ligation buffer (50 mM Tris-HCl pH 7.8/10mM 20 MgCl₂/10mM DTT/1 mM rATP/25 mg/ml bovine serum albumin) at 15°C overnight. The following day the ligation reaction was extracted with phenol/chloroform/isoamyl alcohol (25:24:1). The nucleic acids were ethanol precipitated in the presence of 5 µg of oyster glycogen. The precipitate was dissolved in water and ethanol precipitated again, 25 followed by washing with 70% ethanol. The final pellet was dissolved in 14 µl of water and 1 µl aliquots were electroporated into *E. coli* strain DH-10B (GIBCO-BRL). By this method, a library of approximately 4x10⁶ recombinants was generated.

30 Screening for murine IL-1 Receptor Accessory Protein (muIL-1R AcP) cDNAs by panning with monoclonal antibody 4C5

The panning method has been described previously (Aruffo and Seed, Proc. Natl. Acad. Sci. (USA) 84: 8573, 1987). Ten aliquots from 35 the 3T3-LI library each representing approximately 5x10⁴ clones were plated on LB agar plates containing 100 µg/ml ampicillin (amp) and grown overnight at 37°C. The next day, the colonies from each pool were scraped from the plates into separate 50 ml aliquots of LB +

amp and cultures grown at 37°C for another 2-3 hrs. Plasmid DNA was subsequently extracted using QIAGEN plasmid kits (Qiagen Inc., Chatsworth, CA). The ten separate DNA pools were then used to transfect COS-7 cells by the DEAE dextran technique (5 µg DNA/2x10⁶ cells/9 cm diameter dish) (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press 1989). 72 hrs after transfection, the COS cells were detached from the plates using 0.5 mM EDTA/0.02% Na-azide in phosphate buffered saline (PBS). A single cell suspension was made of each pool. The anti-muIL-1R AcP mAb 4C5 was bound to the cells for 1 hr on ice [(10 µg/ml 4C5 mAb in 3 ml PBS/0.5 mM EDTA/0.02% Na azide/ 5.0% Fetal Calf Serum (FCS)]. The 3 ml of cell-mAb suspension was centrifuged through 6 ml of 2% Ficoll in the above buffer (~300 x g, 5 minutes) to remove unbound mAb. The cells were gently resuspended in the above buffer. The cells from each pool were subsequently added to a single bacterial plate (9 cm diameter) that had been coated with polyclonal goat anti-rat IgG (20 µg/ml in 50 mM Tris-HCl pH 9.5, room temperature, 1.5 hrs) and blocked overnight with PBS/1% BSA at room temperature. COS cells were left on the bacterial plates for 2-3 hrs at room temperature with gentle rocking. Nonadherent cells were gently removed by washing with PBS. The remaining cells were lysed by the addition of 0.8 ml of Hirt lysis solution (0.6% SDS/10 mM EDTA). The lysates were transferred to 1.5 ml Eppendorf tubes and made 1 M NaCl, incubated overnight on ice and spun at 15,000 xg for 15 min at 4°C. The supernatants were extracted with phenol/chloroform/isoamyl alcohol (25:24:1) one time, 10 µg of oyster glycogen was added and the DNA precipitated twice by addition of 0.5 volumes of 7.5 M NH₄OAc and 2.5 volumes of ethanol. The pellet was washed with 70% ethanol, dried and resuspended in 1 µl of H₂O. Each panned pool of DNA was then electroporated into *E. coli* strain DH-10B. After electroporation, 5x10⁴ colonies of each pool were grown as above and plasmid DNA was isolated as above. This DNA represents one round of panning enrichment of the library. A total of three panning rounds were completed keeping each of the ten library pools separate throughout.

After the third round of panning, each of the ten pools was used to transfect COS cells by the DEAE dextran method (1 µg DNA/2x10⁵

- cells/well of a 6-well Costar dish). 72 hrs post transfection, the COS cells were screened for pools that expressed muIL-1R AcP by rosetting with secondary antibody coated polystyrene beads (Dynal Inc., Great Neck, NY). 4C5 mAb was bound to transfected COS cells in PBS/2% FCS (2 µg Ab/well) for 1.5 hrs at room temperature with gentle rocking. Antibody was removed and cells were washed with PBS/2% FCS. 1 ml PBS/2% FCS/1 µl of sheep anti-rat IgG coated polystyrene beads (~4x10⁵ Dynabeads M-450) was added and incubated 1.5 hrs at room temperature with gentle rocking. The beads were removed and the cells washed 5-10 times with PBS. Cells were then fixed by incubation in 95% ethanol/5% acetic acid and examined microscopically for rosetting. One of the ten pools (panning pool #2) was found positive for surface expression of muIL-1R AcP.
- 15 To identify positive clone(s), 100 µl of LB + amp was placed in the wells of two 96-well microtiter plates. Each well was then inoculated with 4 individual colonies from panning pool #2. The bacterial cells were allowed to grow for 5-6 hrs at 37°C. Pools were then made by combining 10 µl aliquots from each well in the 8 rows and 12 columns of each plate, keeping each row and column separate. These pools were each used to inoculate a separate 5 ml culture in LB + amp and grown overnight at 37°C. The next day plasmid DNA was isolated using QIAGEN plasmid kits. Each DNA preparation represented pools of either 48 (rows) or 32 (columns) individual isolates from panning pool #2. Each microtiter pool was used to transfect COS cells in 6-well plates as above and 72 hrs after transfection the cells were screened for Dynabead rosetting as above. Two positive pools were found from one of the microtiter plates, one from row E and one from column 2. A 10 ml aliquot was taken from the well at the intersection of the column and row (well E2) and plated onto LB agar + amp. After overnight incubation, 40 individual colonies were used to each inoculate a 5 ml LB + amp culture. Plasmid DNA was isolated from these cultures using QIAGEN plasmid kits. Each plasmid isolate was digested with *Xba*I restriction enzyme, to release the cDNA insert, and fractionated on a 1.0% agarose gel. This analysis revealed that only three sizes of cDNA inserts were represented in the positive microtiter pool. A single representative of each of the three plasmids was used to transfect COS cells in a 6-well plate as above and screened by

rosetting with Dynabeads. In this way a single cDNA clone (E2-K) was identified that encoded the 4C5-reactive muIL-1R AcP.

Characterization of muIL-1R AcP cDNA's

5

The cDNA clone E2-K (pEF-BOS/muIL-1R AcP) was initially characterized by restriction enzyme mapping. Digestion of this clone with *Xba*I released a 3.2 kilobasepair (kb) cDNA insert. The 3.2 kb *Xba*I fragment was gel-purified and the DNA sequence of both strands was determined by using an ABI automated DNA sequencer along with thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. The DNA sequence revealed an open reading frame (ORF) in the 5-prime half of the clone (see below). Restriction enzyme mapping using Intelligenetics computer software indicated a 1.4 kb *Pst*I restriction fragment within the ORF. This 1.4 kb fragment was gel isolated and used as a probe to identify additional muIL-1R AcP cDNA clones. Approximately 6×10^5 additional clones from the 3T3-LI cDNA library described previously were plated as above. Colony lifts were performed (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press 1989) and the lifts were probed with the 1.4 kb *Pst*I restriction fragment labelled with [³²P]-dCTP by random-priming using the Multiprime DNA labelling system (Amersham Co., Arlington Heights, IL). In this way two additional homologous cDNA clones were isolated. One contained a 1.0 kb insert and the other a 4.3 kb insert as determined by *Xba*I digestion. The DNA sequence of the 4.3 kb insert was determined as above to confirm the sequence of the muIL-1R AcP ORF.

30 Sequence analysis of muIL-1R AcP cDNA clone

The nucleotide sequence of the open reading frame in the muIL-1R AcP cDNA insert is shown in Figure 10A. [SEQ ID NO:4] This open reading frame (ORF) consists of 1710 bp which encodes a protein of 570 amino acids. The amino acid sequence, shown in Figure 10B [SEQ ID NO:6], predicts a 20 amino acid NH₂-terminal signal peptide with cleavage after Ala-1, an extracellular domain from Ser1-Glu339, a hydrophobic transmembrane domain from Leu340-Leu363 and a

cytoplasmic tail from Glu364 to the COOH-terminus. Seven potential N-linked glycosylation sites are all contained within the extracellular domain.

5 Database searches with the protein sequence using the Intelligenetics computer program indicate that muIL-1R AcP has significant homology to both IL-1 Type I and IL-1 Type II receptors from mouse, human, chicken and rat. The homology to each of these proteins is approximately 25% and is uniformly distributed
10 throughout the protein sequence. Further analysis of the amino acid sequence of muIL-1R AcP shows it to be a member of the immunoglobulin superfamily. The three pairs of cysteine residues, conserved in the extracellular domain of all of the IL-1 receptors and responsible for formation of three IgG-like domains, are perfectly
15 conserved in muIL-1R AcP.

Example 8

Mab 4C5 binding to Murine Recombinant IL-1R AcP Expressed in COS
20 cells

To confirm that the cDNA for muIL-1R AcP encodes a protein reactive with mAb 4C5, recombinant muIL-1R AcP was expressed on transfected COS cells and examined for direct binding of [¹²⁵I]-4C5.
25 COS cells were electroporated, by standard methods, with pEF-BOS/muIL-1R AcP. After electroporation, cells were seeded onto a 6 well tissue culture plate at 2-3 x 10⁵ cells/well. After 48-72 hrs growth medium was removed and 1 ml of binding buffer (RPMI/5%FCS) containing 1 x 10⁶ cpm of [¹²⁵I]-4C5 was added per
30 well either alone (total binding) or in the presence of 2 µg unlabelled 4C5 as cold inhibitor (non-specific binding). Both total and non-specific binding were carried out in duplicate. After 3 hrs incubation at 4° C, binding buffer was removed and the cells were washed 3 times with PBS. The cells were then lysed by addition of 0.75 ml of
35 0.5% SDS. The lysates were harvested and bound counts were determined. Specific binding was calculated by subtracting non-specific counts from total counts. Specific counts were approximately 30,000 cpm/ well with a non-specific background of 8% indicating

that pEF-BOS/muIL-1R AcP directs the expression of 4C5 immunoreactive protein in COS cells.

The size of recombinant muIL-1R AcP expressed in COS cells was determined by metabolic labelling of transfected COS cells with [³⁵S]-methionine and immunoprecipitation of labelled muIL-1R AcP with the mAbs 4C5 or 2E6 (Table 2). 36 hrs after electroporation with pEF-BOS/muIL-1R AcP, medium was removed and COS cells were washed 1 time with methionine-free medium [DMEM(high glucose, without methionine-GIBCO-BRL)/10% FBS/1 mM L-glutamine/ 1 mM Na pyruvate)]. Fresh methionine-free medium was added and after 5-8 hrs incubation at 37° C, 50-100 µCi of ³⁵S-methionine was added per ml of medium and incubation continued for 24 hrs. Medium was then removed and the cells washed 2 times with cold PBS. Cells were solubilized by the addition of RIPA buffer (0.5% NP-40, 0.5% Tween-20, 0.5% Deoxycholate, 420mM NaCl, 10mM KCl, 20mM Tris pH 7.5, 1mM EDTA) and incubation on ice for 15 min. The lysate was transferred to tubes and spun at 15,000 x g for 15 min. Lysates were precleared by the addition of 40 µl of GammaBind G Sepharose (50% v/v in RIPA buffer) (Pharmacia Biotech Inc., Piscataway, NJ) to 500 µl of lysate and incubation overnight at 4° C. The next day the precleared lysates were spun 30 sec in a microfuge and lysates were transferred to clean tubes. Another 40 µl of GammaBind G Sepharose was added along with 20 µg mAb 4C5 or 2E6 (Table 2) and the immunoprecipitations were incubated for 3 hrs at 4° C with rotation. The Sepharose-Ab complexes were spun down and washed 1X with RIPA buffer, 1X with 50mM HEPES pH 7.9/200mM NaCl/1mM EDTA/0.5% NP-40 and 1X with 25mM Tris pH 7.5/100mM NaCl/0.5% Deoxycholate/1.0% Triton X-100/0.1% SDS. Protein was released from the beads by addition of 20 µl of 2X Laemmli sample buffer (Laemmli, Nature 227:680, 1970). The proteins were separated by electrophoresis in Tris-Glycine PAGE and visualized by autoradiography. As shown in Figure 11, recombinant muIL-1R AcP immunoprecipitated with mAb 4C5 or 2E6 from transfected COS cells migrates as a broad band from 70-90 kDa. No protein was precipitated from mock transfected COS cells.

Example 9Expression of Recombinant IL-1R AcP in COS Cells: Reactivity with [¹²⁵I]-I-Labeled IL-1 Proteins and Monoclonal Antibodies

5

The binding characteristics of the recombinant IL-1R AcP for [¹²⁵I]-labeled IL-1, 4C5 and 4E2 were determined (Fig. 12). The data showed high level expression of recombinant IL-1R AcP [Cos(4C5)] as determined by [¹²⁵I]-4C5 binding, but no increase in [¹²⁵I]-human IL-1 α binding when compared to control transfected COS cells [Cos(PEF-BOS)]. For comparison, the high level expression of murine recombinant Type I receptor in COS cells [COS (Mu-IL-1R)] as determined by [¹²⁵I]-35F5 binding was accompanied by a corresponding increase in radiolabeled human IL-1 β and IL-1 α binding (Fig. 13).

10

Purification of Natural Murine IL-1 Receptor Accessory Protein (IL-1R
20 AcP) from EL-4 Cells

15

Murine EL-4 cells (100 gm) were solubilized in 1 liter of PBS containing 8 mM CHAPS, 5 mM EDTA and the protease inhibitors pepstatin (10 μ g/ml), leupeptin (10 μ g/ml), benzamidine (1 mM), aprotinin (1 μ g/ml) and PMSF (0.2 mM). After centrifugation at 100,000 x g to remove insoluble material, the supernatant was loaded onto a 50 ml wheat germ agglutinin (WGA) agarose column (Vector Laboratories, Inc.) at 0.8 ml/min. The column was washed with equilibration buffer (PBS, 8 mM CHAPS, 5 mM EDTA) followed by 30 equilibration buffer containing 0.5 M NaCl, and bound protein was eluted with PBS containing 8 mM CHAPS and 0.3 M N-acetyl-D-glucosamine.

25

The sugar-eluted fractions from three WGA agarose column runs were pooled and loaded onto a 5 ml immunoaffinity column [mAb 4C5 antibody cross-linked to Protein G Sepharose via dimethyl-pimelimidate (Stern, A.S. and Podlaski, F.J., in: Techniques in Protein Chemistry IV, R.H. Angeletti, ed., pp. 353-360, Academic Press, NY,

- 1993)] equilibrated with PBS containing 8 mM CHAPS at 1 ml/min. The column was washed with equilibration buffer followed by equilibration buffer containing 1 M NaCl. Bound protein was eluted with 50 mM diethylamine buffer, pH 11.5, containing 8 mM CHAPS.
- 5 The fractions containing IL-1R AcP were dialyzed against PBS containing 4 mM CHAPS and concentrated.

All column fractions were monitored for the presence of IL-1R AcP by SDS-PAGE/immunoblot analysis with mAb 4C5. SDS-PAGE was

10 performed on 8-16% gradient gels (Novex), and proteins were transferred to nitrocellulose as described (Towbin et al., Proc. Natl. Acad. Sci. USA 76: 4350, 1979). After blocking the nitrocellulose with 2.5% casein in 50 mM Tris containing 150 mM NaCl₂ and 0.01%

15 thimerosal (pH 7.5), blots were incubated with mAb 4C5 (5 µg/ml) followed by incubation with HRP-conjugated goat (Fab)₂ anti-rat antibody (Tago Immunologicals). Blots were developed with the ECL System (Amersham Life Science).

The amino acid composition (Hollfelder et al., J. Protein Chem.

20 12: 435, 1993) of the final protein preparation is shown in Table 6; it is similar to the composition predicted from the deduced protein sequence [SEQ ID NO: 3] from the cDNA clone [SEQ ID NO:1] (Figure 16). The remainder of the sample was subjected to SDS-PAGE, transferred to a PVDF membrane (Matsudaira, J. Biol. Chem. 262: 10035, 1987)

25 and stained with Coomassie blue R-250. The protein-stained band at 80 kDa, which was immunoreactive with 4C5 antibody, was analyzed by NH₂-terminal sequence analysis (Hollfelder et al., J. Protein Chem. 12: 435, 1993). Two sequences were obtained (1-3 pmoles of each amino acid per cycle), one of which matched residues 1-12

30 (SERXDDWXLDTM) of the deduced protein sequence obtained from expression cloning of murine IL-1R AcP (Figure 10B).

Although IL-1R AcP solubilized from EL-4 cells has a M_r = 80 kDa as determined by immunoblot analysis with the 4C5 antibody, the

35 predicted molecular weight of the protein from the cDNA sequence is 66 kDa. This apparent difference is likely due to glycosylation of the accessory protein. To address this issue, the affinity purified IL-1R AcP was subjected to SDS-PAGE, and the Coomassie blue-stained band

corresponding to the 80 kDa, 4C5-immunoreactive protein was eluted from the gel and chemically deglycosylated with trifluoromethane sulfonic acid (Edge et al., Anal. Biochem. 118: 131, 1981). The deglycosylated protein migrates with a $M_r = 63\text{-}64$ kDa in SDS-PAGE, 5 a value in good agreement with the predicted molecular weight from the cDNA sequence.

Example 11

10 Isolation of Genomic Clones of Human IL-1 Receptor Accessory Protein

Screening by cross-hybridization

Attempts were made to identify and isolate a cDNA coding for 15 the human homologue of IL-1R AcP by screening human cDNA libraries by

Table 6

Amino Acid Composition of Natural Murine IL-1 Receptor Accessory Protein from EL-4 Cells

amino acid	mole %
Cys	n.d.
Asx	10.5
Thr	5.3
Ser	5.1
Glx	13.1
Pro	n.d.
Gly	8.5
Ala	8.9
Val	7.4
Met	2.7
Ile	6.9
Leu	10.6
Thr	3.3
Phe	4.5
His	2.1
Lys	5.7
Trp	n.d.
Arg	5.4

n.d. = not determined

- 20 cross-hybridization with sequences from murine IL-1R AcP. Human cDNA libraries prepared from mRNA isolated from RAJ1 cells or NC37 cells were probed with the murine IL-1R AcP cDNA, but initial

attempts were unsuccessful, possibly due to very low expression of the human homologue in these cells (see Example 12). We decided to screen a human genomic library to isolate specific sequences that could be used to subsequently screen a human cDNA library.

5

The murine IL-1R AcP cDNA clone [3.2 kb *Xba*I fragment] and restriction fragments of the murine IL-1R AcP cDNA clone [1.4 kb *Pst*I fragment and 843 basepair (bp) *Bam* HI/*Sal*I fragment] were used as probes to perform low-stringency Southern blot analysis of human

10 genomic DNA (Clontech, Palo Alto, CA). This analysis was performed to determine optimal hybridization and washing conditions under which the murine probe could detect homologous sequences present in the human genome. Hybridization with the murine IL-1R AcP-cDNA probes were carried out at 37°C overnight in hybridization buffer A

15 (2X SSC, 20% formamide, 2X Denhardt's, 100 µg/ml yeast RNA, 0.1% SDS). Probes were labelled with [³²P]-dCTP using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The blots were washed with 2X SSC and 0.01% SDS at various temperature points beginning at 37°C. The optimal conditions were determined to be the

20 use of the [³²P]-843 bp *Bam*HI/*Sal*I fragment, hybridizing at 37°C overnight in hybridization buffer A, washing in 2X SSC, 0.01% SDS at 55°C. These conditions yielded the lowest background and were used to screen a commercially available human genomic library.

25 To identify human genomic clones of IL-1R AcP, a human lung fibroblast library in Lambda FIX #944201 (Stratagene, La Jolla, CA) was screened. 4.8 x 10⁵ plaques were screened by standard plaque hybridization techniques (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring

30 Harbor Laboratory Press, 1989) using the conditions described above. Six hybridization positive phage clones were purified by successive plaque hybridization. Two phage clones were further characterized (#1 and #7).

35 Characterization of human genomic clones

The human IL-1R AcP genomic clones were initially characterized by restriction enzyme mapping. Bacteriophage lambda

- DNA was isolated from clones #1 and #7 using LambdaSorb phage adsorbent (Promega, Madison, WI). The phage DNAs were digested with *SacI* to release the inserts, and the fragments were then separated by electrophoresis on 1% agarose gels. Inserts for both 5 clones #1 and #7 were ~17 kb in size. Further mapping of clones #1 and #7 was performed using *XbaI* and *EcoRI*. The digested DNAs were separated by electrophoresis on 1% agarose, transferred to a nylon membrane (ICN, Irvine, CA) and crosslinked for Southern blot analysis. The membrane was hybridized with the 843 bp 10 (*BamHI/SalI*) fragment of murine IL-1R AcP previously described. The probe was labelled with [³²P]-dCTP using Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The blots were hybridized and washed using the low stringency hybridization conditions previously described.
- 15 A 4.5 kb fragment from the *EcoRI* digest and a 2.6 kb fragment from the *XbaI* digest were identified as positive for hybridization to the murine IL-1R AcP sequences. The 4.5 kb fragment and the 2.6 kb fragment were isolated from 0.8% Seaplaque agarose (FMC, Rockland, ME) and purified with Qiaex (Qiagen, Chatsworth, CA). The fragments were subcloned into the vector pBluescript II SK⁺ (Stratagene, La Jolla, CA) to facilitate characterization. Plasmid DNA was prepared using the Qiagen plasmid kit (Qiagen, Chatsworth, CA).
- 25 Southern blot analysis was performed to determine which fragment would be more suitable to detect homologous sequences in the human genome. The 4.5 kb and 2.6 kb fragments were used as probes. Low stringency hybridization conditions were used as follows: 5X SSC, 50% formamide, 5X Denhardt's, 100 µg/ml yeast RNA, 0.1% 30 SDS, 37°C, overnight hybridization. Probes were labelled with [³²P]-d CTP using Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). The membranes were washed using high stringency conditions (0.1 X SSC, 0.01% SDS) at various temperature points beginning at 37°C. Optimal conditions were determined to insure 35 selecting a probe that would be specific for huIL-1R AcP when screening a human cDNA library. These optimal conditions are described in Example 12.

Sequence analysis of human genomic clone

The pBluescript II SK⁺/2.6 kb human genomic IL-1R AcP plasmid DNA was sequenced using an ABI automated DNA sequencer 5 along with thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators. Preliminary DNA sequence analysis showed that this DNA contained a 150-nucleotide region with 90% homology to a sequence coding for the intracellular domain of the murine IL-1R AcP.

10

Example 12Isolation of cDNA Clones of Human IL-1R AcP15 YT cell cDNA library construction

The mAb 2E6 (Example 2, Table 2) was originally characterized by its reactivity with the murine IL-1R AcP. Preliminary data indicated that mAb 2E6 detects the IL-1R AcP on human cells. A 20 number of human cell lines were screened with [¹²⁵I]-2E6 and it was determined that the YT cell line (Yodoi et al., J. Immunol. 134: 1623, 1985) expressed relatively high numbers of 2E6 reactive sites per cell compared to other human cell lines, e.g. RAJI. The YT cell line was therefore chosen as the source of RNA for cDNA library construction.

25

Total RNA was extracted from YT cells and cDNA was made from this RNA as described herein (Example 7: 3T3-LI cDNA library construction). *Eco*RI adapters (Stratagene, La Jolla, CA) were ligated to the resulting cDNAs and molecules >1000 bp were selected by passage 30 over a Sephadryl SF500 column as described herein (EXAMPLE 7: 3T3-LI cDNA library construction). The cDNA was concentrated by ethanol precipitation and ligated to the cloning vector. The cloning vector was Lambda ZAP II phage (Stratagene) that had been digested with *Eco*RI restriction enzyme and dephosphorylated (as provided by the supplier). 10 aliquots of 100ng of size selected cDNA from above were each ligated to 1 µg of Lambda ZAP II arms (*Eco*RI digested and dephosphorylated) in 5 µl of ligation buffer (66 mM Tris-HCl pH 7.5/5mM MgCl₂/1mM DTE/1mM rATP) at 15°C overnight. The

following day the ligations were pooled and packaged into Lambda phage in twelve 4- μ l aliquots using Gigapack II packaging extracts and following the manufacturer's instructions (Stratagene). Packaged phage were titered by plating in bacterial strain XL1-Blue-MRF' 5 (Stratagene) in the presence of 5 mM Isopropyl- β -D-thiogalactopyranoside (IPTG) (Boehringer Mannheim Co., Indianapolis, IN) and 4 mg/ml 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside(X-Gal) (Boehringer-Mannheim) to distinguish non-recombinant phage. Plaque counts the following day indicated that a library of 3.55×10^6 10 recombinants was obtained with a non-recombinant background of <0.1%.

Screening of human cDNA library by hybridization with human genomic clone fragments of IL-1R AcP

15

The 2.6 kb *Xba*I restriction fragment which was previously described as being a specific probe for the huIL-1R AcP was used at low stringency hybridization (5X SSC, 50% formamide, 5X Denhardt's, 100 μ g/ml yeast RNA, 0.1% SDS, 37°C overnight), high stringency wash 20 conditions (0.1X SSC, 0.01% SDS, 40°C) to screen the YT cDNA library. 4.8×10^5 plaques were screened by standard plaque hybridization techniques (Molecular Cloning, A Laboratory Manual, second edition, J. Sambrook, E.I. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press, 1989). Three hybridization positive phage clones (#3, #5, and 25 #6) were identified and purified by successive plaque hybridization. Excision of pBluescript SK (-) phagemids containing insert DNA from the Lambda Zap II vector was performed according to manufacturer's protocol.

30 Characterization of human cDNA clones

The human IL-1R AcP cDNA inserts #3, #5, and #6 in pBluescript SK (-) were further characterized by restriction enzyme mapping. Initially, miniprep plasmid DNA was prepared by the rapid boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). Subsequently, plasmid DNA was prepared with the Qiagen plasmid kit. 35 The plasmid DNAs were digested with *Eco*RI to release the inserts, and the inserts were separated by electrophoresis on 1% agarose. Clone #3

contained a 2.3 kb insert, clone #5 contained a 1.4 kb insert, and clone #6 contained a 2.7 kb insert. Further restriction mapping indicates a single *Pvu*II site present in all three clones.

5 Sequence analysis of human IL-1R AcP cDNA clones

Plasmid DNA from clones #3, #5 and #6 were sequenced using an ABI automated DNA sequencer along with thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators.

- 10 Preliminary sequence analysis indicated that only clones #3 and #6 had inserts that were homologous to the murine IL-1R AcP cDNA. Therefore, clones #3 and #6 inserts were sequenced completely. Sequence analysis indicates that clones #3 and #6 are overlapping clones. Schematic representations of clones #3 and #6 are shown in
- 15 Figure 14. Clone #3 contains the ATG initiation codon and the 5' portion of the coding region. Clone #6 contains the 3' portion of the cDNA and the TGA stop codon. These two overlapping clones were used to construct a full length huIL-1R AcP cDNA.

20

Example 13

Construction of Full Length Human IL-1R AcP cDNA

- Restriction endonuclease mapping and preliminary sequence analysis indicated that there was a single *Bst*XI site present in clone
- 25 #3 and clone #6. Shown in Figure 14 is a schematic representation of overlapping clones #3 and #6. Clones #3 and #6 were digested with the restriction enzymes *Bst*XI and *Xba*I. Fragments of approximately 846 bp and approximately 2700 bp were prepared from clone #3 and clone #6, respectively, by electrophoresis in 0.7% Seaplaque agarose
- 30 (FMC, Rockland, ME) and purified with Qiaex (Qiagen, Chatsworth, CA).

- The full-length human IL-1R AcP was prepared by subcloning into the mammalian expression vector pEF-BOS (Mizushima and Nagato, Nuc. Acids Res. 18: 5322, 1990). pEF-BOS plasmid DNA was
- 35 digested with *Xba*I, treated with calf intestinal phosphatase (Boehringer Mannheim, Indianapolis, IN), separated by electrophoresis on a 0.7% Seaplaque agarose gel, and purified with Qiaex (Qiagen, Chatsworth, CA). The 846 bp and approximately 2700 bp

BstXI/XbaI fragments described above were ligated into the *XbaI*-cleaved pEF-BOS expression vector, and the ligation products were transformed into MC1061 competent cells. The transformed cells were plated onto LB agar plates containing 100 µg/ml ampicillin and grown overnight at 37°C. The next day, 12 individual colonies were picked, inoculated into LB and ampicillin (100 µg/ml) and incubated overnight at 37°C. Miniprep plasmid DNA was prepared from each inoculated colony by the rapid boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). Restriction endonuclease analysis confirmed that 10 clones contained the appropriate insert in the proper orientation relative to the promoter region in pEF-BOS.

Plasmid DNA was isolated from two positive clones #1 and #9 by the Qiagen method (Qiagen, Chatsworth, CA). The nucleotide sequence of both strands of both plasmids was determined as described in Example 7. The sequence of the 1710 bp open reading frame (ORF) contained within the full-length huIL-1R AcP cDNA is shown in Figure 15. [SEQ ID NO:1] The deduced amino acid sequence, shown in Figure 16 [SEQ ID NO:3], would encode a protein of 570 residues consisting of a 20 amino acid signal peptide (Met⁻²⁰-Ala⁻¹), a putative extracellular domain (Ser1-Glu339), a hydrophobic transmembrane domain (Leu340-Leu363), and a cytoplasmic tail (Glu364-Val550). Seven potential N-linked glycosylation sites are all contained within the extracellular domain. All seven sites are conserved between murine and human IL-1R AcP.

Example 14

Expression of Soluble Human IL-1R AcP

30

To express the huIL-1R AcP, a soluble form of the protein was engineered for expression in the baculoviral expression system. This system is useful for overproducing recombinant proteins in eukaryotic cells (Luckow and Summers, Bio/Technology 6: 47, 1988). Using the polymerase chain reaction (PCR) method (Innis M.A., et al., PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego), an amplicon was produced that encoded a soluble form of the extracellular domain of huIL-1R AcP. Briefly, two oligonucleotide

- primers were synthesized on an Applied Biosystems synthesizer. The forward primer contained the *Bam*HI site and the codons for the first 11 amino acids of the signal peptide: (5')GGCC GGA TCC ATG ACA CTT CTG TGG TGT GTA GTG AGT CTC TAC (3') [SEQ ID NO:10]. The reverse 5 primer sequence coded for the 11 amino acids just before the transmembrane domain, an Ala spacer, and a Glu-Glu-Phe tag, followed by the termination codon TAG and a *Kpn*I site: (5') CGCGCG GGT ACC CTA GAA CTC TTC AGC TTC CAC TGT GTA TCT TGG AGC TGG CAC TTT CTGC(3') [SEQ ID NO:11]. The Glu-Glu-Phe tripeptide tag at the 10 COOH-terminus was engineered to provide an epitope for antibody detection of the recombinant protein. This tripeptide tag is recognized by a commercially available monoclonal antibody to α -tubulin (Skinner et al., J. Biol. Chem. 266: 14163, 1991).
- 15 The forward and reverse primers were used to amplify the extracellular domain of the huIL-1R AcP, using clone #3 (Figure 14) as template. The resulting approximately 800 bp PCR amplicon was digested with *Bam*HI and *Kpn*I. The digested fragment was subjected to electrophoresis through 0.7% Seaplaque agarose and purified with 20 Qiaex (Qiagen, Chatsworth, CA). The soluble human IL-1R AcP extracellular domain was then subcloned into pNR1, a derivative of the baculovirus transfer vector pVL941 (PharMingen, San Diego, CA). pNR1 was prepared from pVL941 by removal of the *Eco*RI site at position 7196 (cleavage with *Eco*RI and filling in of sticky ends with 25 Klenow DNA polymerase). The DNA was then subjected to religation, then cleavage with *Bam*HI and *Asp*718 (*Kpn*I isoschizomer) and insertion of the following oligonucleotides which contain *Bam*HI, *Eco*RI, and *Asp*718 recognition sequences:
- 30 (5') GATCCAGAATTCTATAATAG (3') [SEQ ID NO:12]
(3') GTCTTAAGTATTATCCATG (5')[SEQ ID NO:13]

The *Bam*HI, *Eco*RI, and *Asp*718 restriction sites are unique in pNR1.

- 35 pNR1 plasmid DNA was digested with *Bam*HI and *Kpn*I and purified from a 0.7% Seaplaque agarose gel with Qiaex (Qiagen, Chatsworth, CA). The *Bam* HI/*Kpn*I approximately 800 bp huIL-1R AcP PCR amplicon fragment was ligated into the *Bam*HI/*Kpn*I cleaved pNR1 expression vector. The ligation products were transformed into

MC1061 competent cells, which were then plated onto LB agar containing ampicillin (100 µg/ml) and grown overnight at 37°C. The next day, 36 independent colonies were picked and inoculated into LB and ampicillin (100 µg/ml). Miniprep DNA was prepared by the rapid 5 boil method (Holmes and Quigley, Anal. Biochem. 114: 193, 1981). The DNA was analyzed by restriction endonuclease mapping. Thirty plasmid clones were shown to contain the correct insert. Plasmid DNA was prepared from two positive clones (#11, #25) by the Qiagen 10 method (Qiagen, Chatsworth, CA). These clones were verified by sequence analysis.

The pNR1/soluble human IL-1R AcP DNA (clone #25) was co-transfected with linearized AcRP23.lac Z baculovirus DNA (PharMingen, San Diego, CA) into Sf9 (*Spodoptera frugiperda*) cells 15 using the BaculoGold Transfection Kit (PharMingen, San Diego, CA). Following transfection, recombinant baculovirus were isolated and plaque purified according to a protocol described in the BaculoGold Transfection Kit (PharMingen). Plaques were visualized by staining with MTT as described (Shanafelt, Biotechniques 11: 330, 1991). 20 Twelve individual viral plaques were isolated and the virus particles were eluted from the agarose into 0.5 mls of SF-9 media (IPL-41 + 10% FBS - JRH Biosciences, Lenexa, KS) by incubating overnight at 4°C on a rotator. Each recombinant virus was analyzed for the presence of insert by PCR analysis and for the expression of recombinant human 25 IL-1R AcP by immunoblot analysis. For PCR amplification, viral DNA was extracted, incubated with Taq DNA polymerase and the appropriate pNR1 forward and reverse primers (relative to the *Bam*HI/*Asp*718 cloning sites), and amplified using standard PCR methods (Innis *et al.*, PCR Protocols, Academic Press, San Diego 1990). 30 Each amplicon was analyzed by electrophoresis on 1.5% agarose. The results confirmed that 10 out of the 11 plaques tested contained an insert of ~ 1 kb, corresponding to the proper insert size.

For immunoblot analysis, human IL-1R AcP + tag (from the 35 supernatant of Sf9 cells infected with recombinant virus) was isolated by reacting with biotinylated anti-tubulin antibody (YL1/2) (Harlan Bioproducts) immobilized on streptavidin-agarose (Pierce, Rockford, IL). Proteins were eluted from the anti-tubulin antibody matrix with

0.2M glycine pH 2.7, and the fractions neutralized with 3M Tris base. Eluted proteins were treated with Laemmli sample buffer without β -mercaptoethanol, separated on 8% acrylamide (Novex) slab gel and transferred to 0.2 μ nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The immobilized proteins were probed with the YL1/2 antibody (10 μ g/ml), and peroxidase-conjugated goat-anti-rat antibody (1:10,000 dilution) (Boehringer Mannheim Biochemicals). Immunoreactive bands were visualized by ECL (Amersham) according to the manufacturer's protocol. This analysis identified a protein of >200 kDa, that was expressed by recombinant virus containing the human IL-1R AcP + tag insert.

Recombinant virus from plaques #2 and #12 (identified by immunoblot analysis as expressing human IL-1R AcP + tag)were amplified to obtain virus stocks which were used in the large-scale production of human IL-1R AcP + tag for immunization purposes. Sf9 cells were cultured in logarithmic growth (1×10^6 cells/ml) in EX-CELL 401 with 1% Fetal Bovine Serum (JRH Biosciences, Lenexa, KS) at 27°C, infected with recombinant baculovirus as described (O'Reilly *et al.*, Baculovirus Expression Vectors, a Laboratory Manual, Oxford Univ. Press, 1994) and spent culture media were harvested at 3-5 days post-infection. The cells were removed from the spent culture media by centrifugation and the soluble human IL-1R AcP + tag was purified over an affinity matrix composed of immobilized YL1/2 antibody as described in Example 15 below. The purified human IL-1R AcP + tag was used to immunize mice.

Example 15

Preparation and Screening for Monoclonal Antibodies Specific for Human IL-1 Receptor Accessory Protein (huIL-1R AcP)

Three methods are employed to develop antibodies specific for the huIL-1R AcP.

Immunization of mice and rats with COS cells expressing human recombinant IL-1R AcP

COS cells (4×10^7) are transfected by electroporation with the
5 full-length huIL-1R AcP expression plasmid (20 µg, described in Example 13) in a BioRad Gene Pulser at 250 µF and 350 volts as per the manufacturer's protocol. The transfected cells are plated into a 250 mm x 250 mm Nunc tissue culture tray and harvested after 72 hrs growth. The transfected cells are released from the tissue culture tray
10 by treatment with NO-zyme (JRH Biosciences) for 10 min at 37°C. The cells are harvested, washed in PBS, pH 7.4 and used for immunizations. Mice and rats are immunized by the intraperitoneal (i.p.) route with COS cells expressing huIL-1R AcP (1×10^7 cells/animal) on Days 0, 7, 14 and 28. On day 40, the animals are bled to determine the titer of
15 the antibody response against huIL-1R AcP (see below for specific assays). Animals are given booster immunizations (1×10^7 cells, i.p.) at 2-4 week intervals after day 40. Serum antibody titers specific for huIL-1R AcP are determined at 10-12 days after each booster immunization. When the animals develop a sufficient serum antibody
20 titer (e.g., 1/1000 dilution of the serum immunoprecipitates at least 50% of a given amount of the complex of [125 I]-IL-1 β crosslinked to IL-1R AcP solubilized from human YT and RAJI cells), they are given booster immunizations in preparation to isolating their spleen cells. These final booster immunizations are composed of 1×10^7 cells given
25 both i.v. and i.p. on two consecutive days. Three days after the last immunization, spleen cells are isolated from the animal and hybridoma cells are produced as described previously. Hybridoma cells secreting antibodies specific for huIL-1R AcP are identified by the assays described below. Hybridoma cells are cloned as described previously
30 in Example 1.

Immunization of mice and rats with purified human recombinant soluble IL-1R AcP

35 a. Preparation of human recombinant soluble IL-1R AcP in COS cell and baculovirus expression systems. As described above, COS cells are transfected with plasmid DNA expressing the extracellular domain of huIL-1R AcP that has a tag (Glu, Glu, Phe) (Skinner et al., J. Biol.

Chem. 266: 14163, 1991) inserted at the C-terminus (soluble IL-1R AcP, amino acids 1-339 + Ala + Glu + Glu + Phe). The tag encodes the sequence for recognition by the anti-tubulin antibody YL1/2 (Harlan Bioproducts). The medium is harvested from the cells 72 hrs after 5 transfection and soluble IL-1R AcP+tag is detected and purified as described below.

Standard methods (Gruenwald and Heitz, Baculovirus Expression Vector System: Procedures and Methods Manual, Second Edition, 1993, 10 PharMingen, San Diego, CA) are employed to generate a pure recombinant baculovirus expressing the soluble IL-1R AcP protein. Briefly, plasmid DNA coding for the soluble extracellular domain of human IL-1R AcP+tag is inserted into the transfer vector pNR1 as described in Example 14. The recombinant transfer vector is purified 15 and co-transfected with linearized ACVV1.lacZ DNA (PharMingen) into Sf9 (*Spodoptera frugiperda*) cells. Recombinant baculovirus are isolated and plaque-purified. SF-9 cells (2 X 10⁶ cells/ml) are cultured to logarithmic growth phase in TMH-FH medium (PharMingen) at 27°C, infected with recombinant baculovirus, and spent culture media 20 harvested after 3-5 days. The cells are removed from the spent culture media by centrifugation and the soluble IL-1R AcP+tag protein is detected and purified as described below.

b. Preparation of an affinity matrix composed of immobilized YL1/2 antibody. Many methods can be utilized to immobilize the YL1/2 antibody to an affinity matrix including covalent crosslinking to either an activated agarose gel such as Affi-Gel 10 (BioRad Laboratories) or to an agarose gel containing immobilized Protein G (Stern and Podlaski, in: Techniques in Protein Chemistry IV, R.H. 25 Angelletti, ed., pp. 353-360, Academic Press, NY, 1993). However, for the purification of soluble IL-1R AcP, the YL1/2 antibody is covalently modified with NHS-LC-biotin (Pierce Chemical Co.) and immobilized on a streptavidin-agarose gel (Pierce Chemical Co.). YL1/2 antibody (3 mg/ml) is dialyzed against 0.1 M borate buffer, pH 8.5 followed by 30 reaction with NHS-LC-biotin at a molar ratio of 40:1 (LC-biotin:YL1/2 antibody) for 2 hrs at room temperature. The unreacted LC-biotin is quenched with 1 M glycine/0.1 M borate buffer, pH 8.4. The unreacted and quenched NHS-LC-biotin is removed by centrifugation at 1000 xg 35

for 15-30 min using a Centricon-30 microconcentrator (Amicon). After centrifugation, the biotinylated YL1/2 antibody is diluted with 0.1 M sodium phosphate, pH 7.0 and the process repeated two more times. Biotinylated-YL1/2 antibody (6 mg in 0.1 M sodium phosphate, pH 5 7.0) is reacted with streptavidin-agarose (6 ml of a 50% suspension) for 2 hrs at room temperature. The streptavidin agarose with the immobilized biotinylated YL1/2 antibody is placed into a column and washed with 10 column volumes of PBS, pH 7.4.

- 10 c. Purification of soluble IL-1R AcP. Media from either COS cells or Sf9 cells containing soluble IL-1R AcP are passed through the YL1/2 affinity column at a flow rate of 3 ml/min. The column is washed sequentially with 2 column volumes of PBS, pH 7.4, 5 column volumes of 50 mM sodium phosphate, pH 7.5, 0.5 M NaCl, 0.2 % Tween 20, 15 0.05% NaN₃ and 2 column volumes of PBS, pH 7.4. The soluble IL-1R AcP + tag is eluted with 0.1 M glycine-HCL, pH 2.8 and the fractions (1 ml) are neutralized with 3 M Tris base (0.015 ml per 1 ml fraction). The protein eluted from the column (purified soluble IL-1R AcP + tag) is characterized by reducing and non-reducing SDS-PAGE on 12% acrylamide slab gels followed by silver staining to visualize the protein bands. The soluble IL-1R AcP + tag present in the conditioned media from the COS cell and baculovirus expression systems and in the purified preparations can also be identified by western blotting procedures. Proteins in the conditioned media (0.04 ml) and purified 20 soluble IL-1R AcP + tag (0.1 to 1 µg) are treated with Laemmli sample buffer without β-mercaptoethanol, separated by SDS-PAGE on 12% gels and transferred to nitrocellulose membrane (0.2 µM) as described above in Example 1. The proteins immobilized on the nitrocellulose are probed with YL1/2 antibody (5 µg/ml) and peroxidase-conjugated goat 25 anti-murine or -rat IgG antibody (1/1000 dilution) (Boehringer Mannheim Biochemicals). The immunoreactive bands are identified by ECL technique (Amersham Inc.) according to the manufacturer's protocol. The soluble IL-1R AcPs that are purified from COS cell and baculovirus expression systems should migrate as proteins of 30 approximately 65-67 kDa and 45-47 kDa, respectively.

d. Immunization of mice and rats with soluble IL-1R AcP + tag. Mice and rats are immunized by the i.p. and foot pad routes on days 0,

14 and 28 with 10-100 µg of soluble IL-1R AcP + tag. The protein is prepared as described in Examples 1 and 2 in Freund's complete adjuvant for the primary immunization and in Freund's incomplete adjuvant for the day 14 and 28 booster immunizations. Serum is
5 collected from the animals on day 40 and tested for antibody reactivity (see assays below). The animals are given booster immunizations (i.p., 10-25 µg of protein prepared in Freund's incomplete adjuvant) at 4 week intervals and the titer of serum antibodies determined two weeks after each immunization. When the
10 animals develop a potent serum antibody titer (e.g., 1/10⁴ dilution of the serum gives a 50% response in the EIA), they are given booster immunizations (i.v. and i.p.) of 10-100 µg of soluble IL-1R AcP + tag on two consecutive days. Three days later, spleen cells are isolated from the animal and fused with SP2/0 cells as described in Example 1 for
15 the development of the anti-murine IL-1R AcP antibodies. Hybridoma supernatants are screened for inhibitory and non-inhibitory antibodies by the assays described below. Hybridoma cell lines secreting anti-huIL-1R AcP antibodies are cloned by limiting dilution. Anti-huIL-1R AcP antibodies are purified as described in Example 1.

20

e. Assays to detect antibodies specific for human IL-1R AcP. The presence of anti-IL-1R AcP antibodies in the serum is initially determined by enzyme immunoassay (EIA) with soluble IL-1R AcP + tag immobilized on a 96 well plate. Briefly, soluble IL-1R AcP + tag (1 µg/ml) is diluted with 50 mM sodium carbonate buffer, pH 9.0, 0.15 M NaCl (BC saline) and passively adsorbed (100 µl, 100 ng) to the wells of a Nunc Maxisorb plate for 16 hrs at room temperature. After washing, the plates are reacted with PBS, pH 7.4, 1% bovine serum albumin (BSA) for 1 hr at 37°C. Serial dilutions [1/100 to 1/10⁶ in 50 mM
25 sodium phosphate, pH 7.5, 0.5 M NaCl, 0.1% Tween-20, 1% BSA and 0.05% NaN₃ (antibody binding buffer)] of the serum samples are incubated with the immobilized soluble IL-1R AcP for 2 hrs at room temperature. After washing the plate with PBS, pH 7.4, 0.05% Tween-20, the bound antibody is detected with peroxidase-conjugated goat
30 anti-murine or -rat IgG antibody (Boehringer-Mannheim Inc.) and visualized with TMB (tetramethylbenzidine) substrate. The color intensity in the individual wells is measured at 450 nm in a multi-

channel photometer and is proportional to the concentration of anti-IL-1R AcP antibody in the serum.

The serum antibodies are also tested for reactivity by FACS
5 (fluorescence activated cell sorting) on 1) natural huIL-1R AcP expressed on the human cell lines YT, NC-37 and RAJI and 2) recombinant huIL-1R AcP expressed on COS cells. Cells (1×10^6) are incubated with serum dilutions (1/100 to 1/ 10^4) in PBS, pH 7.4 (100 μ l) for 1 hr at 4°C. After washing the cells with PBS, pH 7.4, to remove
10 unbound antibody, the cells are incubated with fluorescein-conjugated goat-anti-mouse or -rat IgG antibody (Tago Laboratories) for 30 min at 4°C. The cells are washed with PBS, pH 7.4, and the quantity of antibody bound to the cell surface is determined by the increase in fluorescence intensity in a FACSort (Becton-Dickinson Co.).

15

The anti-murine IL-1R AcP antibodies 4C5 and 2E6 (Table 2) demonstrated inhibitory and non-inhibitory activity, respectively, against IL-1R AcP expressed on murine cells. To determine if sera from animals immunized with human IL-1R AcP contain both
20 inhibitory and non-inhibitory antibodies, two types of assays are performed: 1) inhibition of [125 I]-IL-1 β binding to human cells and 2) immunoprecipitation of the solubilized complex of [125 I]-IL-1 β crosslinked to cell surface proteins from human cells. For the inhibition assays, serial dilutions of the sera are incubated with YT, NC-37 and
25 RAJI cells ($1-2 \times 10^6$) in binding buffer for 1 hr at room temperature. [125 I]-IL-1 β (25-250 pM) is added to each tube, incubated for 3 hrs at 4°C and cell bound radioactivity determined as previously described in Example 1. The titer of inhibitory antibodies is determined by the serum dilution that results in a 50% decrease in cell-bound
30 radioactivity. For the immunoprecipitation assays, dilutions of serum are incubated for 16 hr at 4°C with the solubilized complexes of [125 I]IL-1 β crosslinked to huIL-1R AcP and in the presence of protein-G-Plus immobilized on agarose beads. Each serum sample is tested for reactivity with solubilized complexes prepared from human
35 cell lines YT, NC-37 and RAJI. After centrifuging and washing the protein-G-Plus agarose beads, the immunoprecipitated proteins are analyzed by SDS-PAGE and autoradiography as described in the Example 1 for the murine IL-1R AcP antibodies.

Immunization of mice and rats with huIL-1R AcP peptides conjugated to keyhole limpet hemocyanin (KLH)

5 Peptides corresponding to sequences 1-10, 54-64, 68-77, 265-
273, 285-294, 490-499 and 505-515 of the full-length huIL-1R AcP
were synthesized by standard solid phase techniques (Marglin and
Merrifield, Ann. Rev. Biochem. 39: 841, 1970). The sequence of each
peptide had a cysteine added to the C-terminus for the purpose of
10 covalent coupling to KLH by the MBS technique. Briefly, KLH (1.5 mg in
PBS, pH 7.4) is reacted with 0.32 mg of 3-maleimidobenzoyl-N-
hydroxy-succinimide ester (MBS; Boehringer Mannheim Biochemicals)
for 1 hr at 4°C. The reaction mix is applied to a prepacked BioGel P10
column (10 ml) (BioRad Laboratories) and chromatographed with PBS,
15 pH 7.4. The fractions containing the KLH-MBS conjugate are pooled (2
ml) and reacted with peptide (2 mg) for 1 hr at 4°C. The KLH-peptide
conjugate is concentrated in a Centricon 10 microconcentrator
(Amicon) and used for immunizations. Mice and rats are immunized by
the i.p. and foot pad routes on day 0, 7, 14 and 28 with 200-500 µg of
20 KLH-peptide conjugate. The conjugate is prepared in Freund's complete
adjuvant for the primary immunization and Freund's incomplete
adjuvant for the booster immunizations. Sera are collected from the
animals on day 40 and tested for antibody reactivity in the soluble IL-
1R AcP EIA. The animals are given booster immunizations (i.p., 100 µg
25 of KLH-peptide conjugate prepared in Freund's incomplete adjuvant)
at 4 week intervals and the titer of serum antibodies determined two
weeks after each immunization. When the animals develop a potent
serum antibody titer ($1/10^4$ dilution gives a 50% response in the EIA),
they are given booster immunizations with free peptide (100 µg, i.v.
30 route) and KLH-peptide conjugate (500 µg, i.p. route) on two
consecutive days. Three days later, spleen cells are isolated from the
animal and hybridoma cells secreting huIL-1R AcP antibodies are
produced and identified as described above.

Example 16Neutralization of IL-1 β Biologic Activity by Anti-Human IL-1R AcP Antibodies and Active Fragments of IL-1R AcP

5 The ability of anti-human IL-1R AcP antibodies to neutralize IL-1 biologic activity in a dose-dependent manner can be determined in the IL-1-induced IL-6 assay with human embryonic lung fibroblast MRC-5 cells (ATCC # CCL-171). MRC-5 cells are plated in 96-well cluster dishes and pretreated for 1 hr with either increasing concentrations of anti-human IL-1R AcP or active fragment of IL-1R AcP. Following the pretreatment, the cells are stimulated with either 5 pM human IL-1 α or IL-1 β for 24 hrs. The amount of IL-6 secreted by the cells in response to IL-1 is measured by a commercially available 10 IL-6 EIA (Quantikine Assay for Human IL-6, R & D Systems, Minneapolis, MN). The inhibitory effects of the antibodies and active fragments of IL-1R AcP are calculated by determining the decrease in 15 IL-6 secretion in the presence and absence of inhibitors. For example, 5 pM and 100 pM IL-1 β stimulated the secretion of approximately 20 8100 and 9800 pg/ml of IL-6, respectively, from MRC-5 cells (Fig. 17). IL-1 receptor antagonist (IL-1RA) and anti-human Type I IL-1R antibody 4C1 blocked this IL-6 secretion in response to IL-1 β (Fig. 17). For IL-1RA and 4C1, the IC₅₀'s for blocking 5 pM IL-1 β were 200 pM and 0.025 μ g/ml, respectively (Fig. 17). The inhibition by IL-1RA 25 and 4C1 can be overridden by increasing the concentration of IL-1 β to 100 pM. With 100 pM IL-1 β , the IC₅₀'s for IL-1RA and 4C1 inhibition were >1 nM and 10 μ g/ml, respectively. These data demonstrated that the IL-1-induced IL-6 response from the MRC-5 cells was 30 specific for IL-1 and a Type I IL-1R-dependent response, in the same way that IL-1-dependent responses in murine cells are also Type I receptor-dependent (Figs. 6, 7 and 8). These IL-1 biologic assays with murine cells led to the identification of neutralizing anti-murine IL-1R AcP antibodies. Similarly, the IL-1 biologic assay with MRC-5 cells 35 can be used to identify neutralizing anti-human IL-1R AcP antibodies and active fragments of IL-1R AcP.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

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- 15 (I) TELEX: 962292/965542 hlr ch

(ii) TITLE OF INVENTION: HUMAN ACCESSORY PROTEIN FOR INTERLEUKIN-1 RECEPTOR

20

(iii) NUMBER OF SEQUENCES: 13

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- 25 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP .

30

(2) INFORMATION FOR SEQ ID NO:1:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1713 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGACACTTC	TGTGGTGTGT	AGTGAGTC	TACTTTATG	GAATCCTGCA	AAGTGATGCC	60
50 TCAGAACGCT	GCGATGACTG	GGGACTAGAC	ACCATGAGGC	AAATCCAAGT	GTTTGAAGAT	120
GAGCCAGCTC	GCATCAAGTG	CCCACCTTT	GAACACTTCT	TGAAATTCAA	CTACAGCACA	180
55 GCCCATTCAAG	CTGGCCTTAC	TCTGATCTGG	TATTGGACTA	GGCAGGACCG	GGACCTTGAG	240
GAGCCAATTAA	ACTTCCGCCT	CCCCGAGAAC	CGCATTAGTA	AGGAGAAAGA	TGTGCTGTGG	300
60 TTCCGGCCCA	CTCTCCTCAA	TGACACTGGC	AACTATAACCT	GCATGTTAAG	GAACACTACA	360
TATTGCAGCA	AAGTTGCATT	TCCCTTGGAA	GTTGTTAAA	AAGACAGCTG	TTTCAATTCC	420

	CCCATGAAAC TCCCAGTGCA TAAACTGTAT ATAGAATATG GCATTCAAGAG GATCACTTGT	480
	CCAAATGTAG ATGGATATTT TCCTTCAGT GTCAAACCGA CTATCACTTG GTATATGGGC	540
5	TGTTATAAAA TACAGAATT TTAAATAATGTA ATACCCGAAG GTATGAACCTT GAGTTTCCTC	600
	ATTGCCTTAA TTTCAAATAA TGGAAATTAC ACATGTGTTG TTACATATCC AGAAAATGGA	660
10	CGTACGTTTC ATCTCACCAAG GACTCTGACT GTAAAGGTAG TAGGCTCTCC AAAAAATGCA	720
	GTGCCCTCTG TGATCCATT ACCTAATGAT CATGTGGTCT ATGAGAAAGA ACCAGGAGAG	780
	GAGCTACTCA TTCCCTGTAC GGTCTATTT AGTTTCTGA TGGATTCTCG CAATGAGGTT	840
15	TGGTGGACCA TTGATGGAAA AAAACCTGAT GACATCACTA TTGATGTCAC CATTAAACGAA	900
	AGTATAAGTC ATAGTAGAAC AGAAGATGAA ACAAGAACTC AGATTTGAG CATCAAGAAA	960
20	GTTACCTCTG AGGATCTCAA GCGCAGCTAT GTCTGTCATG CTAGAAGTGC CAAAGGCAGA	1020
	GTTGCCAAAG CAGCCAAGGT GACGCAGAAA GTGCCAGCTC CAAGATACAC AGTGGAACTG	1080
	GCTTGTGGTT TTGGAGCCAC AGTCCTGCTA GTGGTGATT TCATTGTTGT TTACCATGTT	1140
25	TACTGGCTAG AGATGGTCCT ATTTTACCGG GCTCATTGGA GAACAGATGA AACCAATTAA	1200
	GATGGAAAAG AGTATGATAT TTATGTATCC TATGCAAGGA ATGCGGAAGA AGAAGAATT	1260
30	GTTTTACTGA CCCTCCGTGG AGTTTGGAG AATGAATTG GATACAAGCT GTGCATCTT	1320
	GACCGAGACA GTCTGCCTGG GGGAAATTGTC ACAGATGAGA CTTTGAGCTT CATTCAAGAAA	1380
	AGCAGACGCC TCCTGGTTGT TCTAAGCCCC AACTACGTGC TCCAGGGAAC CCAAGCCCTC	1440
35	CTGGAGCTCA AGGCTGGCCT AGAAAATATG GGCTCTCGGG GCAACATCAA CGTCATTAA	1500
	GTACAGTACA AAGCTGTGAA GGAAACGAAG GTGAAAGAGGC TGAAGAGGGC TAAGACGGTG	1560
	CTCACGGTCA TTAAATGGAA AGGGGAAAAA TCCAAGTATC CACAGGGCAG GTTCTGGAAAG	1620
40	CAGCTGCAGG TGGCCATGCC AGTGAAGAAA AGTCCCAGGC GGTCTAGCAG TGATGAGCAG	1680
	GGCCTCTCGT ATTCACTTTT GAAAAATGTA TGA	1713

45 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1713 base pairs
 (B) TYPE: nucleic acid
 50 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

55 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	TACTGTGAAG ACACCACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCACTACGG	60
	AGTCTTGCAG CGCTACTGAC CCCTGATCTG TGGTACTCCG TTTAGGTTCA CAAACTTCTA	120
5	CTCGGTCGAG CGTAGTTCAC GGGTGAGAAA CTTGTGAAGA ACTTTAAGTT GATGTCGTGT	180
	CGGGTAAGTC GACCGGAATG AGACTAGACC ATAACCTGAT CCGTCCTGGC CCTGGAACTC	240
	CTCGGTTAAT TGAAGGCGGA GGGGCTCTTG GCGTAATCAT TCCTCTTCT ACACGACACC	300
10	AAGGCCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTTC CTTGTGATGT	360
	ATAACGTCGT TTCAACGTAAGGGAACCTT CAACAAGTTT TTCTGTCGAC AAAGTTAAGG	420
15	GGGTACTTTG AGGGTCACGT ATTTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA	480
	GGTTTACATC TACCTATAAA AGGAAGGTCA CAGTTGGCT GATAGTGAAC CATATACCCG	540
	ACAATATTTT ATGTCTTAAA ATTATTACAT TATGGGCTTC CATACTTGAA CTCAAAGGAG	600
20	TAACGGAATT AAAGTTTATT ACCTTTAATG TGTACACAAC AATGTATAGG TCTTTACCT	660
	GCATGCAAAG TAGAGTGGTC CTGAGACTGA CATTTCATC ATCCGAGAGG TTTTTACGT	720
25	CACGGGGGAC ACTAGGTAAG TGGATTACTA GTACACCAGA TACTCTTCT TGTCCTCTC	780
	CTCGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA	840
	ACCACCTGGT AACTACCTTT TTTGGACTA CTGTAGTGAT AACTACAGTG GTAATGCTT	900
30	TCATATTCAAG TATCATCTTG TCTTCTACTT TGTTCTTGAG TCTAAAACTC GTAGTTCTTT	960
	CAATGGAGAC TCCTAGAGTT CGCGTCGATA CAGACAGTAC GATCTTCACG GTTCCGCTT	1020
35	CAACGGTTTC GTCGGTTCCA CTGCGTCTTT CACGGTCGAG GTTCTATGTG TCACCTTGAC	1080
	CGAACACCAA AACCTCGGTG TCAGGACGAT CACCACTAAG AGTAACAACA AATGGTACAA	1140
	ATGACCGATC TCTACCAGGA TAAAATGGCC CGAGTAAAAC CTTGTCTACT TTGGTAAAAT	1200
40	CTACCTTTTC TCATACTATA AATACATAGG ATACGTTCT TACGCCTCTC TCTTCTTAAA	1260
	CAAAATGACT GGGAGGCACC TCAAAACCTC TTACTTAAAC CTATGTTCGA CACGTAGAAA	1320
45	CTGGCTCTGT CAGACGGACC CCCTTAACAG TGTCTACTCT GAAACTCGAA GTAAGTCTTT	1380
	TCGTCTGCGG AGGACCAACA AGATTGGGG TTGATGCACG AGGTCCCTTG GGTTGGGAG	1440
	GACCTCGAGT TCCGACCGGA TCTTTATAC CCGAGAGCCC CGTTGTAGTT GCAGTAAAAT	1500
50	CATGTCATGT TTGACACTT CCTTGCTTC CACTTCTCG ACTTCTCCCG ATTCTGCCAC	1560
	GAGTGCCAGT AATTACCTT TCCCCTTTTT AGGTTCATAG GTGTCCCGTC CAAGACCTTC	1620
55	GTCGACGTCC ACCGGTACGG TCACCTCTT TCAGGGTCCG CCAGATCGTC ACTACTCGTC	1680
	CCGGAGAGCA TAAGTAGAAA CTTTTTACAT ACT	1713

(2) INFORMATION FOR SEQ ID NO:3:

- 75 -

	Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys			
	245	250	255	
5	Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe			
	260	265	270	
	Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys			
	275	280	285	
10	Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His			
	290	295	300	
	Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys			
	305	310	315	320
15	Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser			
	325	330	335	
20	Ala Lys Gly Glu Val Ala Lys Ala Lys Val Thr Gln Lys Val Pro			
	340	345	350	
	Ala Pro Arg Tyr Thr Val Glu Leu Ala Cys Gly Phe Gly Ala Thr Val			
	355	360	365	
25	Leu Leu Val Val Ile Leu Ile Val Val Tyr His Val Tyr Trp Leu Glu			
	370	375	380	
	Met Val Leu Phe Tyr Arg Ala His Phe Gly Thr Asp Glu Thr Ile Leu			
	385	390	395	400
30	Asp Gly Lys Glu Tyr Asp Ile Tyr Val Ser Tyr Ala Arg Asn Ala Glu			
	405	410	415	
35	Glu Glu Glu Phe Val Leu Leu Thr Leu Arg Gly Val Leu Glu Asn Glu			
	420	425	430	
	Phe Gly Tyr Lys Leu Cys Ile Phe Asp Arg Asp Ser Leu Pro Gly Gly			
	435	440	445	
40	Ile Val Thr Asp Glu Thr Leu Ser Phe Ile Gln Lys Ser Arg Arg Leu			
	450	455	460	
	Leu Val Val Leu Ser Pro Asn Tyr Val Leu Gln Gly Thr Gln Ala Leu			
	465	470	475	480
45	Leu Glu Leu Lys Ala Gly Leu Glu Asn Met Gly Ser Arg Gly Asn Ile			
	485	490	495	
50	Asn Val Ile Leu Val Gln Tyr Lys Ala Val Lys Glu Thr Lys Val Lys			
	500	505	510	
	Glu Leu Lys Arg Ala Lys Thr Val Leu Thr Val Ile Lys Trp Lys Gly			
	515	520	525	
55	Glu Lys Ser Lys Tyr Pro Gln Gly Arg Phe Trp Lys Gln Leu Gln Val			
	530	535	540	
	Ala Met Pro Val Lys Lys Ser Pro Arg Arg Ser Ser Ser Asp Glu Gln			
	545	550	555	560
60	Gly Leu Ser Tyr Ser Ser Leu Lys Asn Val			
	565	570		

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 5 (A) LENGTH: 1713 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA
 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
 ATGGGACTTC TGTGGTATTG GATGAGTCTG TCCTTCTATG GGATCCTGCA GAGTCATGCT 60

20 TCGGAGCGCT GTGATGACTG GGGACTAGAT ACCATGCGAC AAATCCAAGT GTTTGAAGAT 120
 GAGCCGGCTC GAATCAAGTG CCCCCCTCTTT GAACACTTCC TGAAGTACAA CTACAGCACT 180
 25 GCCCATTCCCT CTGGCCTTAC CCTGATCTGG TACTGGACCA GGCAAGACCG GGACCTGGAG 240
 GAGCCCATTG ACTTCCGCCT CCCAGAGAAT CGCATCAGTA AGGAGAAAAGA TGTGCTCTGG 300
 30 TTCCGGCCCA CCCTCCTCAA TGACACGGGC AATTACACCT GCATGTTGAG GAACACAAC 360
 TACTGCAGCA AAGTTGCATT TCCCCTGGAA GTTGTTCAGA AGGACAGCTG TTTCAATTCT 420
 GCCATGAGAT TCCCAGTGCA CAAGATGTAT ATTGAACATG GCATTCTAA GATCACATGT 480
 35 CCAAATGTAG ACGGATACTT TCCTTCCAGT GTCAAACCAT CGGTCACTTG GTATAAGGGT 540
 TGTACTGAAA TAGTGGACTT TCATAATGTA CTACCCGAGG GCATGAACCTT GAGCTTTTC 600
 40 ATCCCCTTGG TTTCAAATAA CGGAAATTAC ACATGTGTGG TTACATATCC TGAAAACGGA 660
 CGTCTCTTTC ACCTCACCAAG GACTGTGACT GTAAAGGTGG TGGGCTCACC AAAGGATGCA 720
 TTGCCACCCC AGATCTATTG TCCAAATGAC CGTGTGTCT ATGAGAAAAGA ACCAGGAGAG 780
 45 GAACTGGTTA TTCCCTGCAA AGTCTATTTC AGTTTCATTA TGGACTCCCA CAATGAGGTC 840
 TGGTGGACCA TTGATGGAAA GAAGCCTGAT GACGTCACAG TCGACATCAC TATTAATGAA 900
 50 AGTGTAAAGTT ATTCTTCAAC GGAAGATGAA ACAAGGACTC AGATTTGAG CATCAAGAAA 960
 GTCACCCCGG AGGATCTCAG GCGCAACTAT GTCTGTCATG CTCGAAATAC CAAAGGGAA 1020
 GCTGAGCAGG CTGCCAAGGT GAAACAGAAA GTCATACCCAC CAAGGTACAC AGTAGAACTC 1080
 55 GCCTGTGGTT TTGGAGCCAC GGTCTTCTG GTAGTGGTTC TCATTGTGGT TTACCATGTT 1140
 TACTGGCTGG AGATGGTCCT CTTTTACCGA GCTCACTTTG GAACAGATGA AACAAATTCTT 1200
 60 GATGGAAAGG AGTATGATAT TTATGTTCC TATGCAAGAA ATGTGGAAGA AGAGGAATT 1260
 GTGCTGCTGA CGCTGCGTGG AGTTTGGAG AATGAGTTG GATACAAGCT GTGCATCTTC 1320
 GACAGAGACA GCCTGCCTGG GGGATTGTC ACAGATGAGA CCCTGAGCTT CATTCAAGAAA 1380

	AGCAGACGAC TCCTGGTTGT CCTAAGTCCC AACTACGTGC TCCAGGGAAC ACAAGCCCTC	1440
5	CTGGAGCTCA AGGCTGGCCT AGAAAATATG GCCTCCGGG GCAACATCAA CGTCATTTA	1500
	GTGCAGTACA AAGCTGTGAA GGACATGAAG GTGAAAGAGC TGAAGCGGGC TAAGACGGTG	1560
	CTCACGGTCA TTAAATGGAA AGGAGAGAAA TCCAAGTATC CTCAGGGCAG GTTCTGGAAG	1620
10	CAGTTGCAGG TGGCCATGCC AGTGAAGAAG AGTCCCAGGT GGTCTAGCAA TGACAAGCAG	1680
	GGTCTCTCCT ACTCATCCCT GAAAAACGTA TGA	1713

(2) INFORMATION FOR SEQ ID NO:5:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1713 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: cDNA
- 25 (iii) HYPOTHETICAL: NO
- 25 (iv) ANTI-SENSE: YES

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

	TACCCCTGAAG ACACCATAAA CTACTCAGAC AGGAAGATAAC CCTAGGACGT CTCAGTACGA	60
35	AGCCTCGGA CACTACTGAC CCCTGATCTA TGGTACGCTG TTTAGGTTCA CAAACTTCTA	120
	CTCGGCCGAG CTTAGTTCAC GGGGGAGAAA CTTGTGAAGG ACTTCATGTT GATGTCGTGA	180
	CGGGTAAGGA GACCGGAATG GGACTAGACC ATGACCTGGT CCGTTCTGGC CCTGGACCTC	240
40	CTCGGGTAAT TGAAGGCGGA GGGTCTCTA GCGTAGTCAT TCCTCTTTCT ACACGAGACC	300
	AAGGCCGGT GGGAGGGAGTT ACTGTGCCCG TTAATGTGGA CGTACAACTC CTTGTGTTGA	360
45	ATGACGTGTT TTCAACGTAA AGGGGACCTT CAACAAGTCT TCCTGTCGAC AAAGTTAAGA	420
	CGGTACTCTA AGGGTCACGT GTTCTACATA TAACTGTAC CGTAAGTATT CTAGTGTACA	480
	GGTTTACATC TGCCTATGAA AGGAAGGTCA CAGTTGGTA GCCAGTGAAC CATATTCCA	540
50	ACATGACTTT ATCACCTGAA AGTATTACAT GATGGGCTCC CGTACTTGAA CTCGAAAAAG	600
	TAGGGGAACC AAAGTTTATT GCCTTTAATG TGTACACACC AATGTATAGG ACTTTTGCCT	660
55	GCAGAGAAAG TGGAGTGGTC CTGACACTGA CATTCCACC ACCCGAGTGG TTTCTACGT	720
	AACGGTGGGG TCTAGATAAG AGGTTTACTG GCACAACAGA TACTCTTTCT TGGTCCTCTC	780
	CTTGACCAAAT AAGGGACGTT TCAGATAAG TCAAAGTAAT ACCTGAGGGT GTTACTCCAG	840
60	ACCACCTGGT AACTACCTTT CTTGGACTA CTGCAGTGTG AGCTGTAGTG ATAATTACTT	900
	TCACATTCAA TAAGAAGTTG CTTCTACTT TGTTCTGAG TCTAAAACTC GTAGTTCTTT	960

	CAGTGGGCC	TCCTAGAGTC	CGCGTTGATA	CAGACAGTAC	GAGCTTATG	GTTTCCCCCTT	1020
	CGACTCGTCC	GACGGTTCCA	CTTGTCTTT	CAGTATGGTG	GTTCCATGTG	TCATCTTGAG	1080
5	CGGACACCAA	AACCTCGGTG	CCAGAAAAGAC	CATCACCAAG	AGTAACACCA	AATGGTACAA	1140
	ATGACCGACC	TCTACCAGGA	AAAAATGGCT	CGAGTGAAAC	CTTGTCTACT	TTGTTAAGAA	1200
10	CTACCTTCC	TCATACTATA	AATACAAAGG	ATACGTTCTT	TACACCTTCT	TCTCCTTAAA	1260
	CACGACGACT	GCGACGCACC	TCAAAACCTC	TTACTCAAAC	CTATGTTCGA	CACGTAGAAG	1320
	CTGTCTCTGT	CGGACGGACC	CCCTTAACAG	TGTCTACTCT	GGGACTCGAA	GTAAGTCTTT	1380
15	TCGTCTGCTG	AGGACCAACA	GGATTCAAGG	TTGATGCACG	AGGTCCCTTG	TGTTGGGAG	1440
	GACCTCGAGT	TCCGACCGGA	TCTTTATAC	CGGAGGGCCC	CGTTGTAGTT	GCAGTAAAAT	1500
20	CACGTCACTGT	TTCGACACTT	CCTGTACTTC	CACTTTCTCG	ACTTCGCCCG	ATTCTGCCAC	1560
	GAGTGCCTAGT	AATTTACCTT	TCCTCTCTTT	AGGTTCACTAG	CAGTCCCGTC	CAAGACCTTC	1620
	GTCAACGTCC	ACCGGTACGG	TCACTTCTTC	TCAGGGTCCA	CCAGATCGTT	ACTGTTCGTC	1680
25	CCAGAGAGGA	TGAGTAGGGGA	CTTTTTGCA	ACT			1713

(2) INFORMATION FOR SEQ ID NO:6:

- 30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 570 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (iii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

45 Met Gly Leu Leu Trp Tyr Leu Met Ser Leu Ser Phe Tyr Gly Ile Leu
1 5 10 15

Gln Ser His Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met
20 25 30

50 Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro
35 40 45

55 Leu Phe Glu His Phe Leu Lys Tyr Asn Tyr Ser Thr Ala His Ser Ser
50 55 60

Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu
 65 70 75 80

60 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys
85 90 95

	Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr			
	100	105	110	
5	Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro			
	115	120	125	
	Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Ala Met Arg Phe			
	130	135	140	
10	Pro Val His Lys Met Tyr Ile Glu His Gly Ile His Lys Ile Thr Cys			
	145	150	155	160
	Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Ser Val Thr			
	165	170	175	
15	Trp Tyr Lys Gly Cys Thr Glu Ile Val Asp Phe His Asn Val Leu Pro			
	180	185	190	
20	Glu Gly Met Asn Leu Ser Phe Phe Ile Pro Leu Val Ser Asn Asn Gly			
	195	200	205	
	Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Leu Phe His			
	210	215	220	
25	Leu Thr Arg Thr Val Thr Val Lys Val Val Gly Ser Pro Lys Asp Ala			
	225	230	235	240
	Leu Pro Pro Gln Ile Tyr Ser Pro Asn Asp Arg Val Val Tyr Glu Lys			
	245	250	255	
30	Glu Pro Gly Glu Glu Leu Val Ile Pro Cys Lys Val Tyr Phe Ser Phe			
	260	265	270	
	Ile Met Asp Ser His Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys			
35	275	280	285	
	Pro Asp Asp Val Thr Val Asp Ile Thr Ile Asn Glu Ser Val Ser Tyr			
	290	295	300	
40	Ser Ser Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys			
	305	310	315	320
	Val Thr Pro Glu Asp Leu Arg Arg Asn Tyr Val Cys His Ala Arg Asn			
	325	330	335	
45	Thr Lys Gly Glu Ala Glu Gln Ala Ala Lys Val Lys Gln Lys Val Ile			
	340	345	350	
50	Pro Pro Arg Tyr Thr Val Glu Leu Ala Cys Gly Phe Gly Ala Thr Val			
	355	360	365	
	Phe Leu Val Val Val Leu Ile Val Val Tyr His Val Tyr Trp Leu Glu			
	370	375	380	
55	Met Val Leu Phe Tyr Arg Ala His Phe Gly Thr Asp Glu Thr Ile Leu			
	385	390	395	400
	Asp Gly Lys Glu Tyr Asp Ile Tyr Val Ser Tyr Ala Arg Asn Val Glu			
	405	410	415	
60	Glu Glu Glu Phe Val Leu Leu Thr Leu Arg Gly Val Leu Glu Asn Glu			
	420	425	430	

	Phe	Gly	Tyr	Lys	Leu	Cys	Ile	Phe	Asp	Arg	Asp	Ser	Leu	Pro	Gly	Gly
	435							440						445		
5	Ile	Val	Thr	Asp	Glu	Thr	Leu	Ser	Phe	Ile	Gln	Lys	Ser	Arg	Arg	Leu
	450				455				460							
	Leu	Val	Val	Leu	Ser	Pro	Asn	Tyr	Val	Leu	Gln	Gly	Thr	Gln	Ala	Leu
	465				470				475						480	
10	Leu	Glu	Leu	Lys	Ala	Gly	Leu	Glu	Asn	Met	Ala	Ser	Arg	Gly	Asn	Ile
	485							490						495		
	Asn	Val	Ile	Leu	Val	Gln	Tyr	Lys	Ala	Val	Lys	Asp	Met	Lys	Val	Lys
	500							505						510		
15	Glu	Leu	Lys	Arg	Ala	Lys	Thr	Val	Leu	Thr	Val	Ile	Lys	Trp	Lys	Gly
	515							520						525		
20	Glu	Lys	Ser	Lys	Tyr	Pro	Gln	Gly	Arg	Phe	Trp	Lys	Gln	Leu	Gln	Val
	530							535						540		
	Ala	Met	Pro	Val	Lys	Lys	Ser	Pro	Arg	Trp	Ser	Ser	Asn	Asp	Lys	Gln
	545							550						555		560
25	Gly	Leu	Ser	Tyr	Ser	Ser	Leu	Lys	Asn	Val						
	565															

(2) INFORMATION FOR SEQ ID NO:7:

- | | | |
|----|-------|---------------------------|
| 30 | (i) | SEQUENCE CHARACTERISTICS: |
| | (A) | LENGTH: 1077 base pairs |
| | (B) | TYPE: nucleic acid |
| | (C) | STRANDEDNESS: single |
| | (D) | TOPOLOGY: linear |
| 35 | (ii) | MOLECULE TYPE: cDNA |
| | (iii) | HYPOTHETICAL: NO |
| 40 | (iv) | ANTI-SENSE: NO |

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

45	ATGACACTTC	TGTGGTGTGT	AGTGAGTCTC	TACTTTATG	GAATCCTGCA	AAAGTGATGCC	60
	TCAGAACGCT	GCGATGACTG	GGGACTAGAC	ACCATGAGGC	AAATCCAAGT	GTTTGAAGAT	120
50	GAGCCAGCTC	GCATCAAGTG	CCCACTCTTT	GAACACTTCT	TGAAATTCAA	CTACAGCACA	180
	GCCCATTCA	CTGGCCTTAC	TCTGATCTGG	TATTGGACTA	GGCAGGACCG	GGACCTTGAG	240
	GAGCCAATTA	ACTTCCGCCT	CCCCGAGAAC	CGCATTAGTA	AGGAGAAAAGA	TGTGCTGTGG	300
55	TTCCGGCCA	CTCTCCTCAA	TGACACTGGC	AACTATAACCT	GCATGTTAAG	GAACACTACA	360
	TATTGCAGCA	AAGTTGCATT	TCCCTTGGAA	GTGTTCAAA	AAGACAGCTG	TTTCAATTCC	420
60	CCCATGAAAC	TCCCAGTGCA	TAAACTGTAT	ATAGAATATG	GCATTCAGAG	GATCACTTGT	480
	CCAAATGTAG	ATGGATATT	TCCTTCCAGT	GTCAAACCGA	CTATCACTTG	GTATATGGGC	540

	TGTTATAAAA TACAGAATTT TAATAATGTA ATACCCGAAG GTATGAAC TT GAGTTTCCTC	600
	ATTGCCTTAA TTTCAAATAA TGGAAATTAC ACATGTGTTG TTACATATCC AGAAAATGGA	660
5	CGTACGTTTC ATCTCACCAAG GACTCTGACT GTAAAGGTAG TAGGCTCTCC AAAAAATGCA	720
	GTGCCCTCTG TGATCCATTAC ACCTAATGAT CATGTGGTCT ATGAGAAAGA ACCAGGAGAG	780
	GAGCTACTCA TTCCCTGTAC GGTCTATTTT AGTTTCTGA TGGATTCTCG CAATGAGGTT	840
10	TGGTGGACCA TTGATGGAAA AAAACCTGAT GACATCACTA TTGATGTCAC CATTAAACGAA	900
	AGTATAAGTC ATAGTAGAAC AGAAGATGAA ACAAGAACTC AGATTTGAG CATCAAGAAA	960
15	GTTACCTCTG AGGATCTCAA GCGCAGCTAT GTCTGTCATG CTAGAAGTGC CAAAGGCAGAA	1020
	GTTGCCAAAG CAGCCAAGGT GACGCAGAAA GTGCCAGCTC CAAGATACAC AGTGGAA	1077
	(2) INFORMATION FOR SEQ ID NO:8:	
20	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 1077 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
25	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
	(iii) HYPOTHETICAL: NO	
30	(iv) ANTI-SENSE: YES	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:	
	TACTGTGAAG ACACCACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCAC TACGG	60
	AGTCTTGCGA CGCTACTGAC CCCTGATCTG TGGTACTCCG TTTAGGTTCA CAAACTTCTA	120
40	CTCGGTCGAG CGTAGTTCAC GGGTGAGAAA CTTGTGAAGA ACTTTAAGTT GATGTCGTGT	180
	CGGGTAAGTC GACCGGAATG AGACTAGACC ATAACCTGAT CCGTCCTGGC CCTGGAACTC	240
45	CTCGGTTAAT TGAAGGCGGA GGGGCTCTTG GCGTAATCAT TCCTCTTTCT ACACGACACC	300
	AAGGCCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTG CTTGTGATGT	360
	ATAACGTCGT TTCAACGTA AGGGAACCTT CAACAAGTTT TTCTGTCGAC AAAGTTAAGG	420
50	GGGTACTTTG AGGGTCACGT ATTTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA	480
	GGTTTACATC TACCTATAAA AGGAAGGTCA CAGTTGGCT GATAGTGAAC CATATAACCG	540
55	ACAATATTTC ATGTCTTAAA ATTATTACAT TATGGCTTC CATACTTGAA CTCAAAGGAG	600
	TAACGGAATT AAAGTTTATT ACCTTTAATG TGTACACAAC AATGTATAGG TCTTTTACCT	660
	GCATGCAAAG TAGAGTGGTC CTGAGACTGAA CATTCCATC ATCCGAGAGG TTTTTTACGT	720
60	CACGGGGGAC ACTAGGTAAG TGGATTACTA GTACACCAGA TACTCTTTCT TGGTCCTCTC	780
	CTCGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA	840

5	ACCACTGGT AACTACCTTT TTTTGGACTA CTGTAGTGAT AACTACAGTG GTAATTGCTT	900
	TCATATTCAG TATCATCTTG TCTTCTACTT TGTTCTTGAG TCTAAAACTC GTAGTTCTTT	960
	CAATGGAGAC TCCTAGAGTT CGCGTCGATA CAGACAGTAC GATCTTCACG GTTTCCGCTT	1020
	CAACGGTTTC GTCGGTTCCA CTGCGTCTTT CACGGTCGAG GTTCTATGTG TCACCTT	1077

10 (2) INFORMATION FOR SEQ ID NO:9:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 359 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

 (iii) HYPOTHETICAL: NO

 (iv) ANTI-SENSE: NO

- 83 -

Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly
 195 200 205

5 Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His
 210 215 220

Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala
 225 230 235 240

10 Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys
 245 250 255

Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe
 15 260 265 270

Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys
 275 280 285

20 Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His
 290 295 300

Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys
 305 310 315 320

25 Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser
 325 330 335

Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Thr Gln Lys Val Pro
 30 340 345 350

Ala Pro Arg Tyr Thr Val Glu
 355

(2) INFORMATION FOR SEQ ID NO:10:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCCGGATCC ATGACACTTC TGTGGTGTGT AGTGAGTCTC TAC

43

(2) INFORMATION FOR SEQ ID NO:11:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 61 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

10 CGCGCGGGTA CCCTAGAACT CTTCAGCTTC CACTGTGTAT CTTGGAGCTG GCACCTTCTG 60
C 61

(2) INFORMATION FOR SEQ ID NO:12:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: NO

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
GATCCAGAAC TCATAATAG 19

(2) INFORMATION FOR SEQ ID NO:13:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
GTCTTAAGTA TTATCCATG 19

Claims

1. A polynucleotide which encodes an IL-1 receptor accessory protein or an active fragment thereof.
5
2. A polynucleotide of claim 1 comprising a DNA sequence selected from
 - (a) a polynucleotide having essentially the sequence [SEQ ID NO:1]; or
10
 - (b) a polynucleotide which hybridizes to the DNA of (a) under moderately stringent conditions; or
 - (c) a polynucleotide which differs in codon sequence due to the degeneracy of the genetic code.
- 15 3. A polynucleotide of claim 1 or claim 2 which encodes a human IL-1 receptor accessory protein.
4. A polynucleotide of claim 3 which encodes the human IL-1 receptor protein having the amino acid sequence [SEQ ID NO:3] or an
20 active fragment thereof.
5. A polynucleotide of claim 4 having the sequence [SEQ ID NO:1]
- 25 6. A polynucleotide of claim 1 or claim 2 which encodes a soluble IL-1 receptor accessory protein.
7. A polynucleotide of claim 6 which encodes a human
30 soluble IL-1 receptor accessory protein.
8. A polynucleotide of claim 7 which encodes the human soluble IL-1 receptor protein having the amino acid sequence [SEQ ID NO:9] or an active fragment thereof.
35
9. A polynucleotide of claim 8 having the sequence [SEQ ID NO:7].

10. A polynucleotide of claim 1 or claim 2 which is an antisense polynucleotide.
- 5 11. A vector which comprises a polynucleotide according to any of claims 1 to 10.
12. A vector of claim 11 which is an expression vector.
- 10 13. A host cell which comprises a vector of claim 11 or claim 12.
14. The IL-1 receptor accessory protein or an active fragment thereof.
- 15 15. A protein of claim 14 encoded by a polynucleotide as defined in claim 2.
16. A protein according to claim 14 or claim 15 which is the 20 human IL-1 receptor accessory protein.
17. A protein of claim 16 which has the amino acid sequence [SEQ ID NO:3].
- 25 18. A protein according to claim 14 or claim 15 which is a soluble human IL-1 receptor accessory protein.
19. A protein of claim 18 having the amino acid sequence [SEQ ID NO:9].
- 30 20. A protein according to any of claims 14 to 19 carrying one or more side groups which have been modified.
21. An antibody which binds specifically to the human IL-1 35 receptor accessory protein and prevents activation of the IL-1 receptor complex by IL-1.
22. An antibody of claim 21 which is a monoclonal antibody.

23. An antibody according to claim 21 or claim 22 having a binding affinity to the IL-1 receptor accessory complex of from about KD 0.1 nM to about KD 10 nM.

5 24. A pharmaceutical composition which comprises a compound according to any of claims 10 and 14 to 23 and a pharmaceutically acceptable carrier.

10 25. A pharmaceutical composition according to claim 24 in combination with one or more other cytokine antagonists.

26. A process for the preparation of an IL-1 receptor accessory protein comprising the steps of:

- 15 (a) expressing a polypeptide encoded by a DNA according to any of claims 1 to 10 in a suitable host,
(b) isolating said IL-1 receptor accessory protein, and
(c) if desired, converting it in an analogue wherein one or more side groups are modified.

20 27. A process for the preparation of an IL-1 receptor accessory protein antibody comprising the steps of:

- 25 (a) preparation of a hybridoma cell line producing a monoclonal antibody which specifically binds to the IL-1 receptor accessory protein and
(b) production and isolation of the monoclonal antibody.

28. A compound as claimed in any one of claims 14 to 23 prepared by a process as claimed in claim 26 or claim 27.

30 29. A compound according to any of claims 10 and 14 to 23 for use as therapeutically active substance.

35 30. A compound according to any of claims 10 and 14 to 23 for use in the treatment of inflammatory or immune responses and/or for regulating and preventing inflammatory or immunological activities of Interleukin-1.

31. A compound according to any of claims 10 and 14 to 23 in the treatment of acute or chronic diseases, preferably rheumatoid arthritis, inflammatory bowel disease, septic shock, transplant 5 rejection, psoriasis, asthma and Type I diabetes or in the treatment of cancer, preferably acute and chronic myelogenous leukemia.

32. The use of a compound according to any of claims 10 and 14 to 23 for the manufacture of a medicament for the control or 10 prevention of illness.

33. The use of a compound of claim 10 and 14 to 23 for the manufacture of a medicament for the treatment of inflammatory or immune responses and/or for regulating and preventing 15 inflammatory or immunological activities of Interleukin-1.

34. The use of a compound of claims 10 and 14 to 23 for the manufacture of a medicament for the treatment or prophylaxis of rheumatoid arthritis, inflammatory bowel disease, septic shock, 20 transplant rejection, psoriasis, asthma and Type I diabetes or for the treatment or prophylaxis of cancer, preferably acute and chronic myelogenous leukemia.

35. The novel compounds, compositions, processes and uses 25 thereof substantially as described herein.

Fig. 1

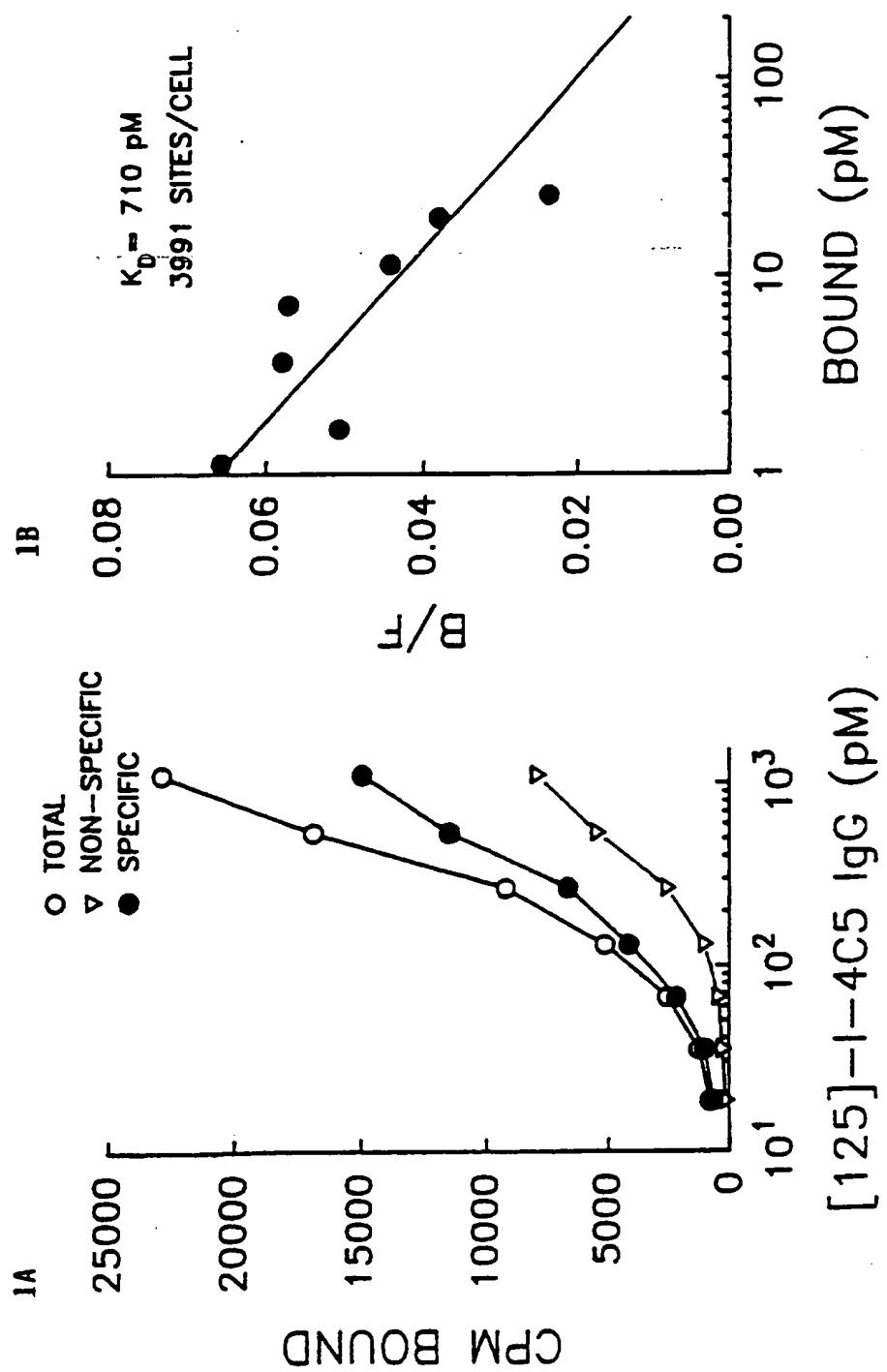
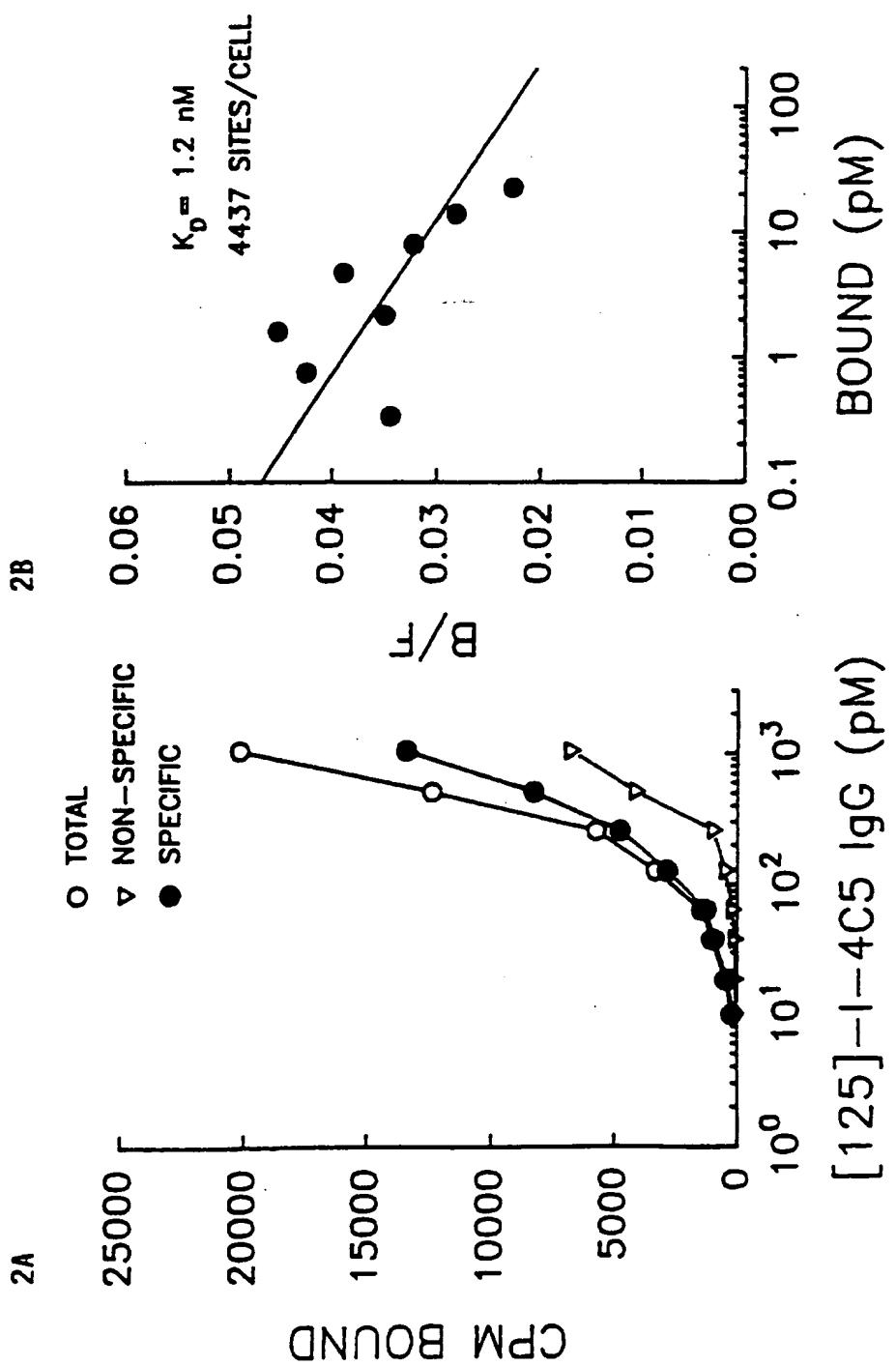
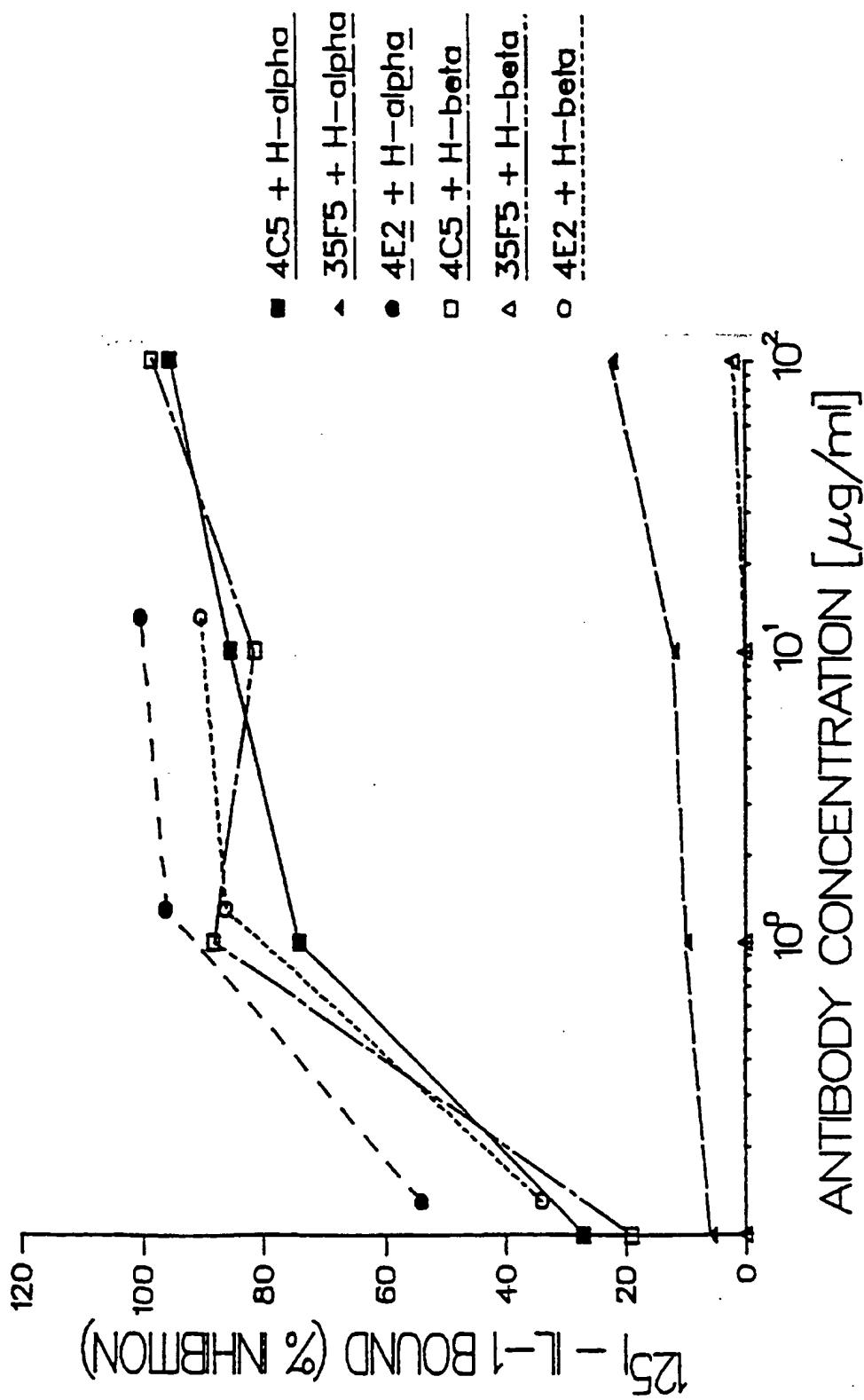


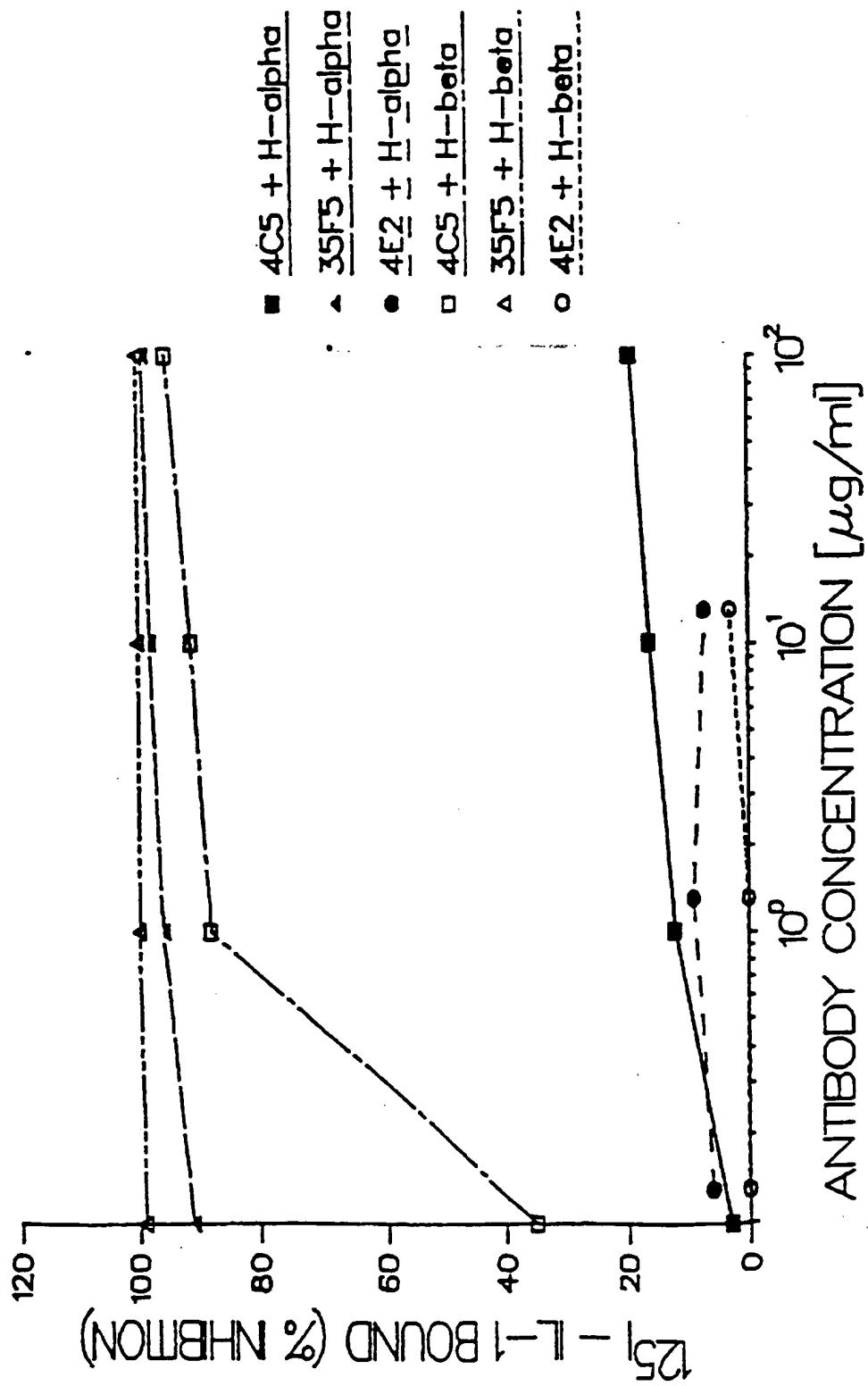
Fig. 2

3/23

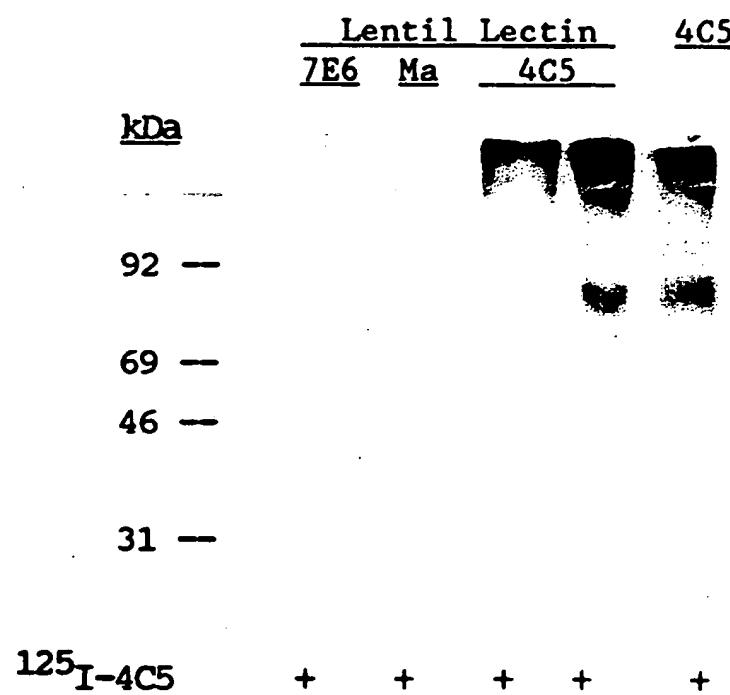
Fig. 3



4/23

Fig. 4

5/23

Fig. 5

6/23

Fig. 6A

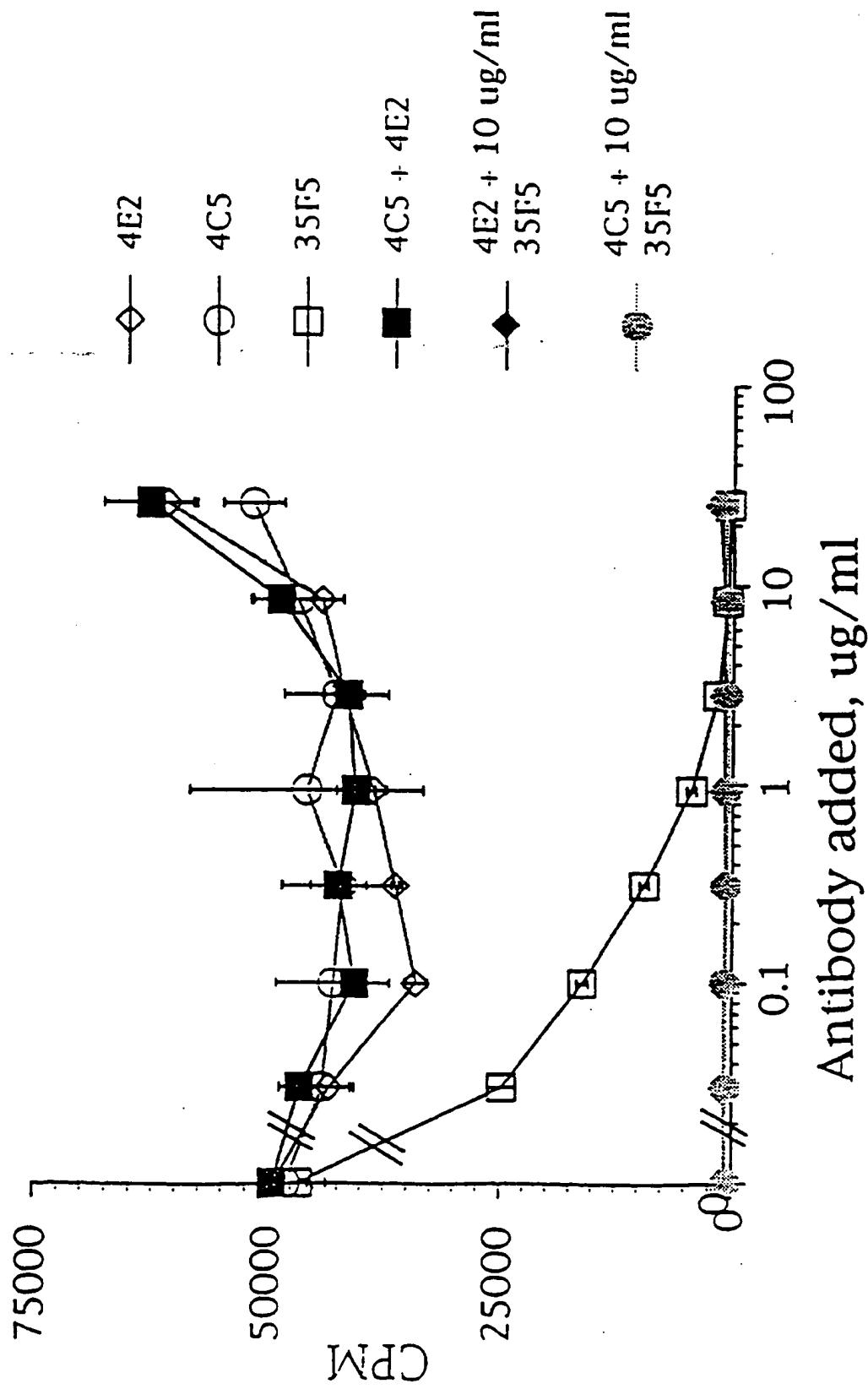
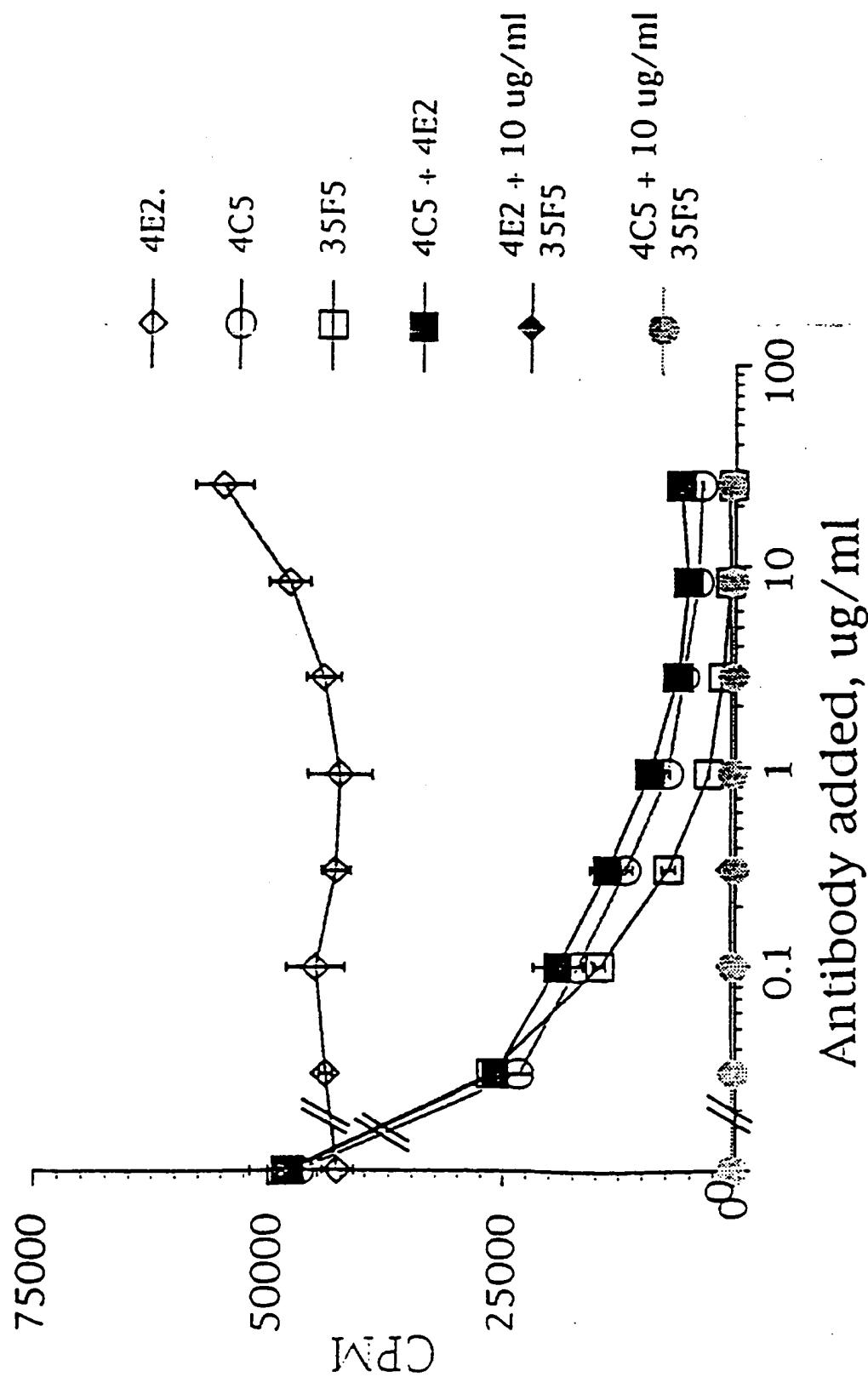
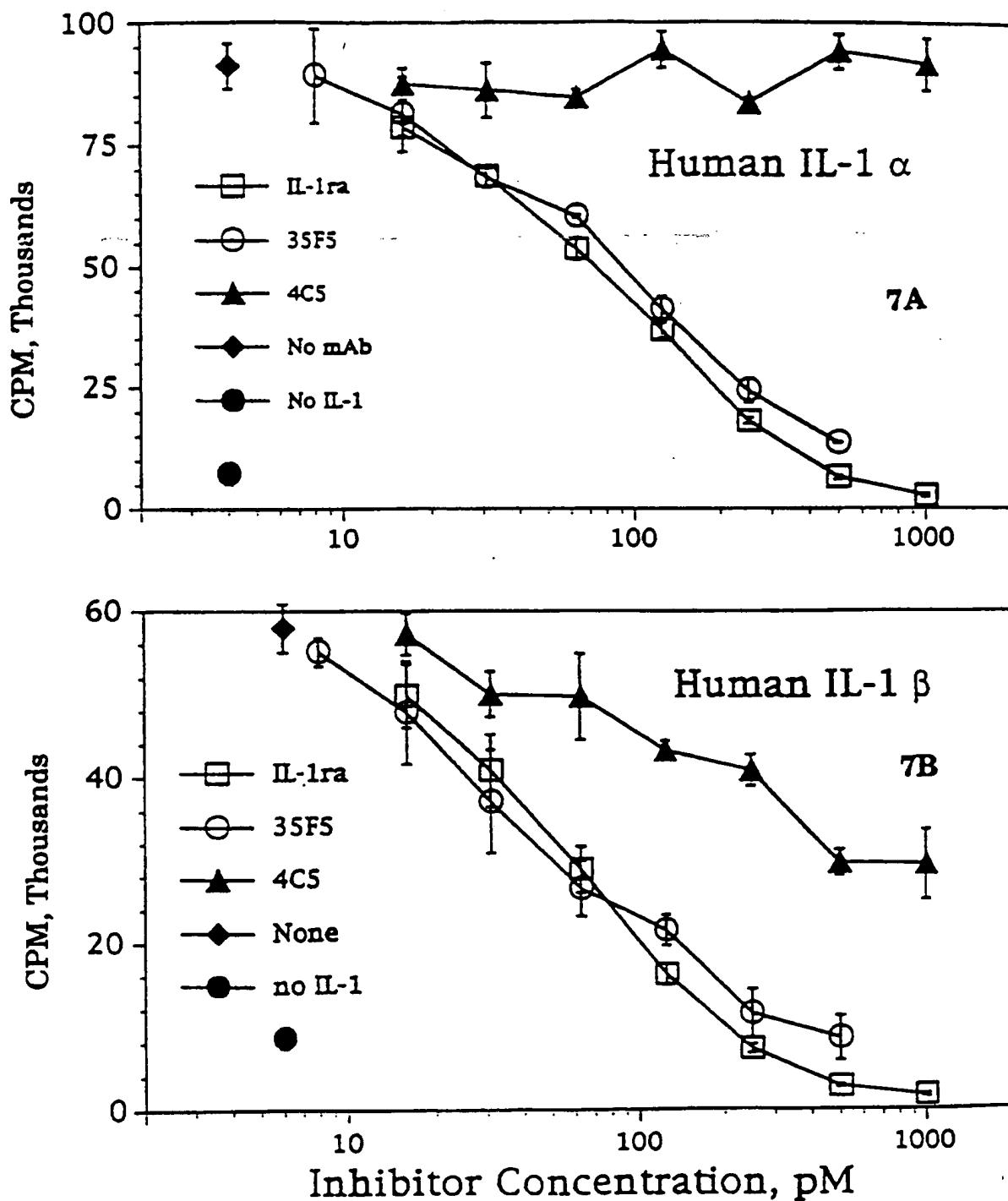


Fig. 6B

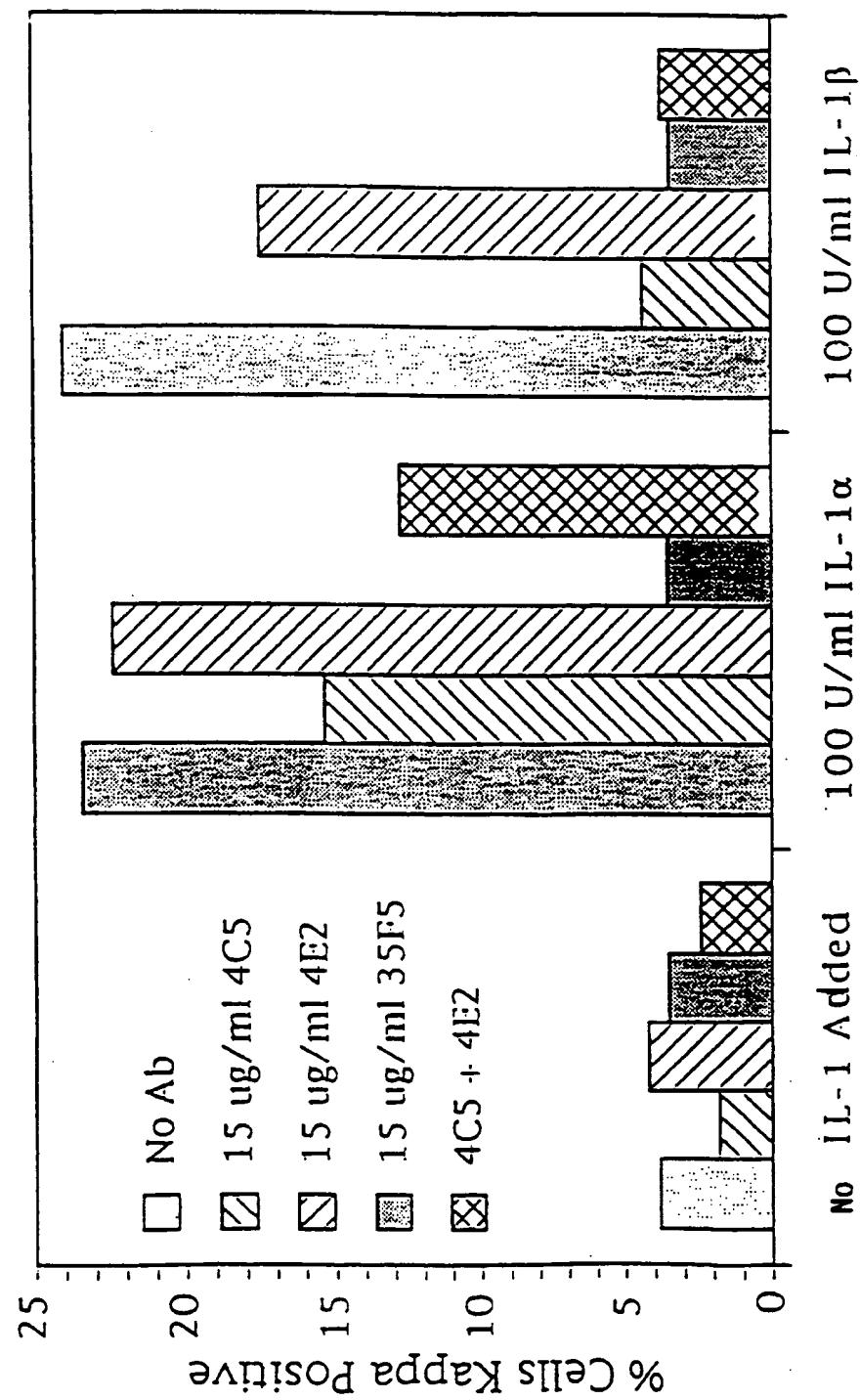
7/23



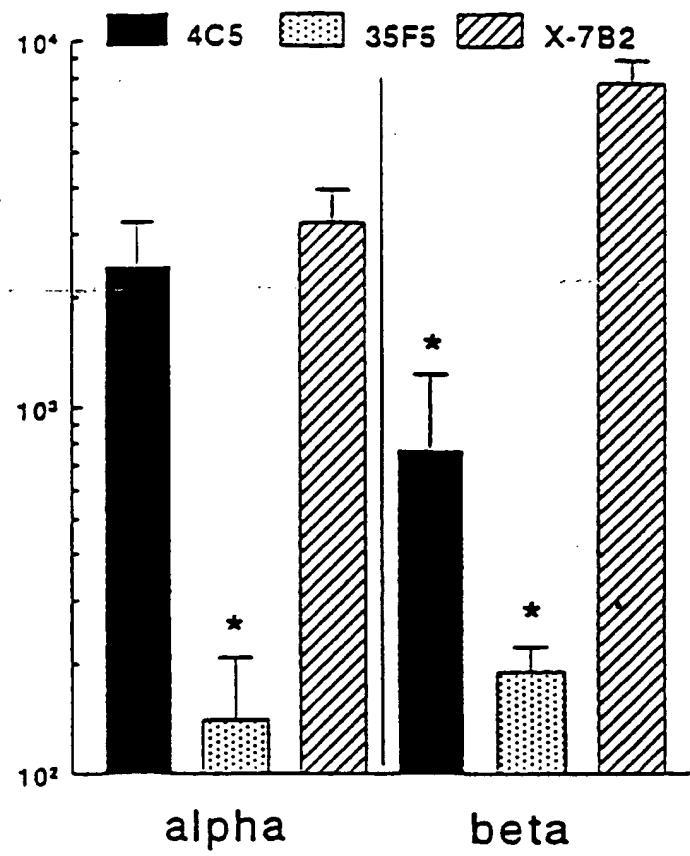
8/23

Fig. 7

9/23

Fig. 8

10/23

Fig. 9

11/23

Fig. 10A

10	20	30	40	50	60	70
ATGGGACTTC	TGTGGTATTT	GATGAGTCTG	TCCTTCTATG	GGATCCTGCA	GAGTCATGCT	TCGGAGCGCT
TACCCCTGAAG	ACACCATAAA	CTACTCAGAC	AGGAAGATAAC	CCTAGGACGT	CTCAGTACGA	AGCCTCGCGA
80	90	100	110	120	130	140
GTGATGACTG	GGGACTAGAT	ACCATCGGAC	AAATCCAAGT	GTTCGAAGAT	GAGCCGGCTC	GAATCAAGTG
CACTACTGAC	CCCTGATCTA	TGGTACGCTG	TTTAGGTTCA	CAAACCTCTA	CTCGGCCGAG	CTTAGTTCAC
150	160	170	180	190	200	210
CCCCCTCTTT	GAACACTTCC	TGAAGTACAA	CTACAGCACT	GCCCATTCT	CTGGCCTTAC	CCTGATCTGG
GGGGGAGAAA	CTTGTGAAGG	ACTTCATGTT	GATGTCGTGA	CGGGTAAGGA	GACCGGAATG	GGACTAGAAC
220	230	240	250	260	270	280
TACTGGACCA	GGCAAGACCG	GGACCTGGAG	GAGCCCATT	ACTTCGGCCT	CCCAGAGAAAT	CGCATCAGTA
ATGACCTGGT	CCGTTCTGGC	CCTGGACCTC	CTCGGGTAAT	TGAAGGCGGA	GGGTCTCTTA	GCGTAGTCAT
290	300	310	320	330	340	350
AGGAGAAAGA	TGTGCTCTGG	TTCCGGCCCA	CCCTCCTCAA	TGACACGGGC	AATTACACCT	GCATGTTGAG
TCCTCTTTCT	ACACGGAGACC	AAGGCCGGGT	GGGAGGAGTT	ACTGTGCCCG	TTAATGTGGA	CGTACAACTC
360	370	380	390	400	410	420
GAACACAACT	TACTGCAGCA	AAGTTGCATT	TCCCCTGGAA	GTGTTTCAGA	AGGACAGCTG	TTTCAATTCT
CTTGTGTTGA	ATGACGTCGT	TTCAACGTAA	AGGGGACCTT	CAAACAGTCT	TCCTGTCGAC	AAAGTTAAGA
430	440	450	460	470	480	490
GCCATGAGAT	TCCCAGTGCA	CAAGATGTAT	ATTGAACATG	GCATTCTAA	GATCACATGT	CCAAATGTPG
CGGTACTCTA	AGGGTCACGT	GTTCTACATA	TAACTTGTAC	CGTAAGTATT	CTAGTGTACA	GGTTTACATC
500	510	520	530	540	550	560
ACGGATACTT	TCCTTCCAGT	GTCAAACCAT	CGGTCACTTG	GTATAAGGGT	TGTACTGAAA	TAGTGGACTT
TGCCTATGAA	AGGAAGGTCA	CAGTTGGTA	GCCAGTGAAC	CATATCCCCA	ACATGACTTT	ATCACCTGAA
570	580	590	600	610	620	630
TCATAATGTA	CTACCCGGAGG	GCATGAACTT	GAGCTTTTC	ATCCCCTTGG	TTTCAAATAA	CGGAAATTAC
AGTATTACAT	GATGGGCTCC	CGTACTTGAA	CTCGAAAAAAG	TAGGGGAACC	AAAGTTTATT	GCCTTTAATG
640	650	660	670	680	690	700
ACATGTGTTG	TTACATATCC	TGAAAACGGA	CGTCTCTTTC	ACCTCACCAAG	GACTGTGACT	GTAAAGGTGG
TGTACACACC	AATGTATAGG	ACTTTTGCT	GCAGAGAAAG	TGGAGTGGTC	CTGACACTGA	CATTCCACC
710	720	730	740	750	760	770
TGGGCTACC	AAAGGATGCA	TTGCCACCCC	AGATCTATTTC	TCCAAATGAC	CGTGTGTTCT	ATGAGAAAAGA
ACCCGAGTGG	TTTCTACGT	AACGGTGGGG	TCTAGATAAG	AGGTTTACTG	GCACAACAGA	TACTCTTCT
780	790	800	810	820	830	840
ACCAGGAGAG	GAACCTGGTTA	TTCCCTGCAA	AGTCTATTTC	AGTTTCATTA	TGGACTCCCA	CAATGAGCTC
TGGTCCTCTC	CTTGACCAAT	AAGGGACGTT	TCAGATAAAAG	TCAAAGTAAT	ACCTGAGGGT	GTACTCCAG
850	860	870	880	890	900	910
TGGTGGACCA	TTGATGGAAA	GAAGCCTGAT	GACGTACAG	TCGACATCAC	TATTAATGAA	AGTGTAAAGTT
ACCACCTGGT	AACTACCTTT	CTTCGGACTA	CTGCACTGTC	AGCTGTAGTG	ATAATTACTT	TCACATTCAA

12/23

Fig. 10A cont.

920 930 940 950 960 970 980
 ATTCTTCAC GGAAGATGAA ACAAGGACTC AGATTTTGTAG CATCAAGAAA GTCACCCCCGG AGGATCTCAG
 TAAGAAGTTG CCTTCTACTT TGTTCTGAG TCTAAAACTC GTAGTTCTTT CAGTGGGCC TCCTAGAGTC

 990 1000 1010 1020 1030 1040 1050
 GCGCAACTAT GTCTGTCAATG CTCGAAATAC CAAAGGGGAA GCTGAGCAGG CTGCCAAGGT GAAACAGAAA
 CGCGTTGATA CAGACAGTAC GAGCTTATG GTTCCCTT CGACTCGTCC GACGGTTCCA CTTTGTCTTT

 1060 1070 1080 1090 1100 1110 1120
 GTCATACCAC CAAGGTACAC AGTAGAACTC GCCTGTGGTT TTGGAGGCCAC GGTCTTTCTG GTAGTGGTC
 CAGTATGGTG GTTCCATGTG TCATCTTGTAG CGGACACCAA AACCTCGGTG CCAGAAAGAC CATCACCAAG

 1130 1140 1150 1160 1170 1180 1190
 TCATTGTGGT TTACCATGTT TACTGGCTGG AGATGGCTCT CTTTACCGA GCTCACTTTG GAACAGATGA
 AGTAACACCA AATGGTACAA ATGACCGACC TCTACCAAGGA GAAAATGGCT CGAGTGAAAC CTTGTCTACT

 1200 1210 1220 1230 1240 1250 1260
 ACAATTCTT GATGAAAGG AGTATGATAT TTATGTTTCC TATGCAAGAA ATGTGGAAGA AGAGGAATTT
 TTGTTAAGAA CTACCTTCC TCATACTATA AATACAAAGG ATACGTTCTT TACACCTTCT TCTCCTTAAA

 1270 1280 1290 1300 1310 1320 1330
 GTGCTGCTGA CGCTGCGTGG AGTTTGTGAG AATGAGTTTG GATACAAGCT GTGCATCTTC GACAGAGACA
 CACGACGACT GCGACGCACC TCAAAACCTC TTACTCAAAC CTATGTTGCA CACGTAGAAG CTGTCTCTGT

 1340 1350 1360 1370 1380 1390 1400
 GCCTGCCTGG GGGAAATTGTC ACAGATGAGA CCTTGAGCTT CATTCAAGAAA AGCAGACGAC TCCGGTTGT
 CGGACGGACC CCCTTAACAG TGTCTACTCT GGGACTCGAA GTAAGTCTTT TCGTCTGCTG AGGACCAACA

 1410 1420 1430 1440 1450 1460 1470
 CCTAAAGTCCC AACTACGTGCA TCCAGGGAAC ACAAGCCCTC CTGGAGCTCA AGGCTGGCCT AGAAAATATG
 GGATTCAAGG TTGATGCACG AGGTCCCTTG TGTCTGGAG GACCTCGAGT TCCGACCGGA TCTTTATAC

 1480 1490 1500 1510 1520 1530 1540
 GCCTCCCGGG CCAACATCAA CGTCATTITA GTGCACTACA AAGCTGTGAA CGACATGAAG GTGAAAGAGC
 CGGAGGGCCC CGTTGTAGTT GCAGTAAAT CACGTCACTGT TTGACACTT CCTGTACTTC CACTTTCTCG

 1550 1560 1570 1580 1590 1600 1610
 TGAAGCGGGC TAAGACGGTG CTCACGGTCA TTAAATGGAA AGGAGAGAAA TCCAAGTATC CTCAGGGCAG
 ACTTCGCCCC ATTCTGCCAC GAGTGCCAGT AATTACCTT TCCCTCTTT AGGTTCATAG GAGTCCCGTC

 1620 1630 1640 1650 1660 1670 1680
 GTTCTGGAAG CAGTTCAGG TGGCCATGCC AGTGAAGAAG AGTCCCAGGT GGTCTAGCAA TGACAAGCAG
 CAAGACCTTC GTCAACGTCC ACCGGTACGG TCACTCTTC TCAGGGTCCA CCAGATCGTT ACTGTTGTC

 1690 1700 1710
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 CCAGAGAGGA TGAGTAGGGA CTTTTGCGAT ACT

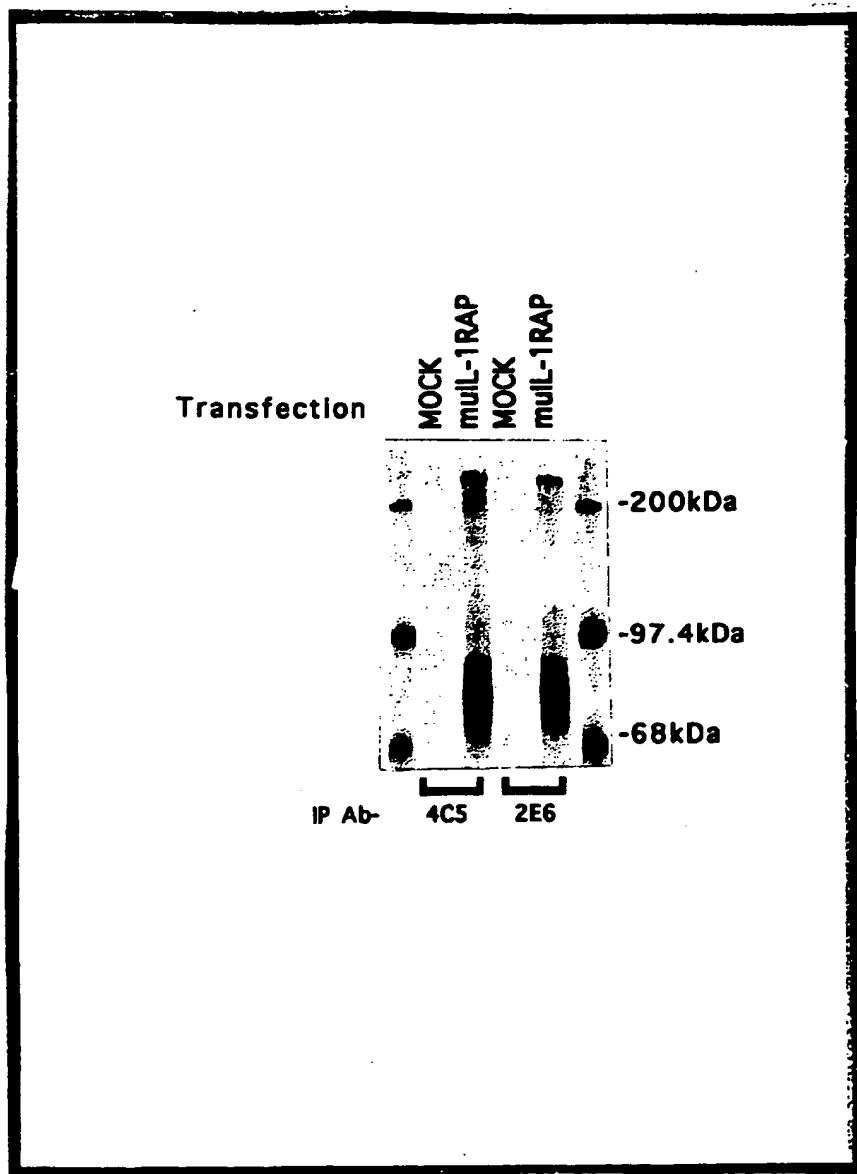
13/23

Fig. 10 B

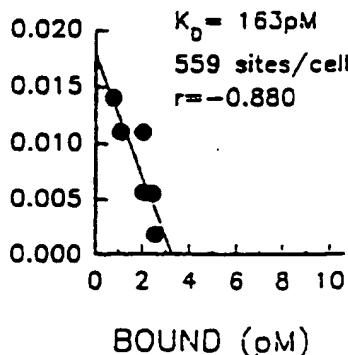
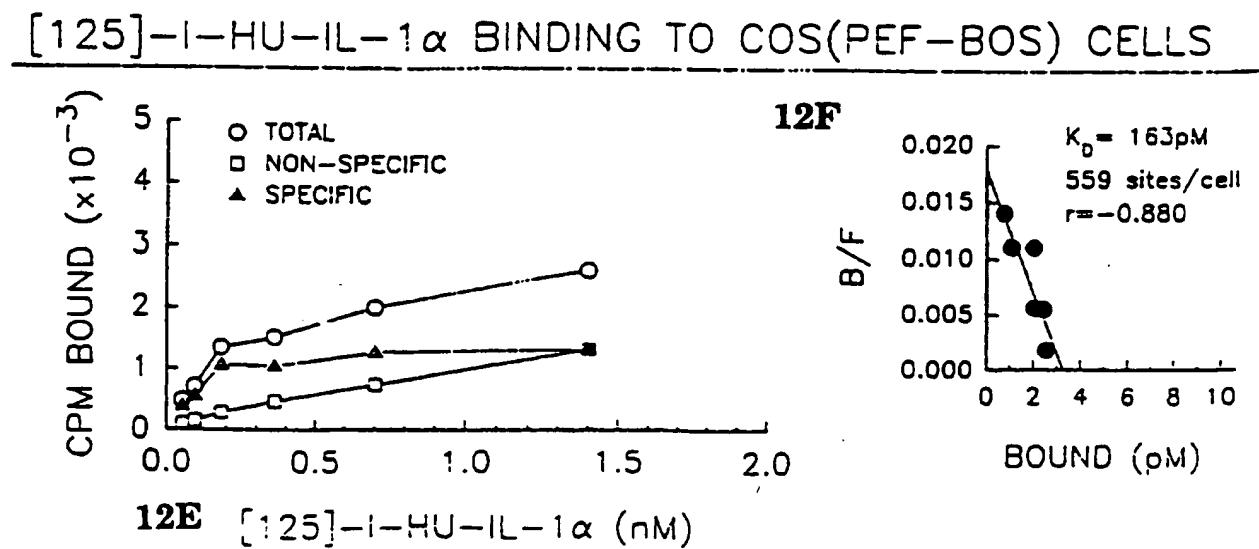
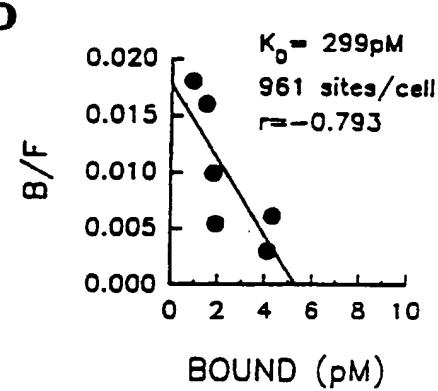
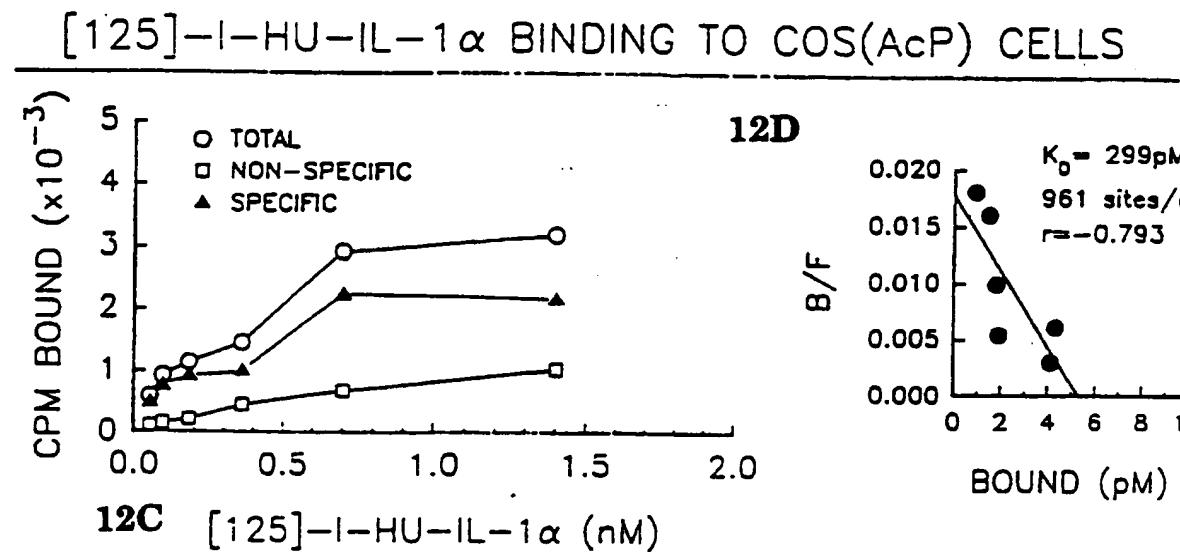
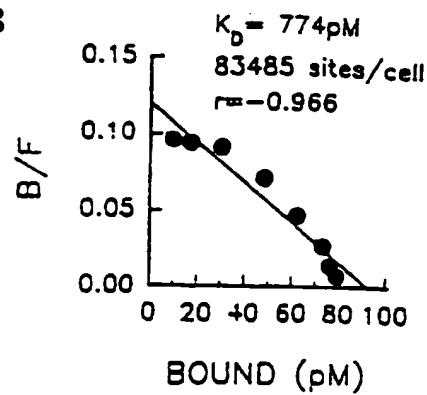
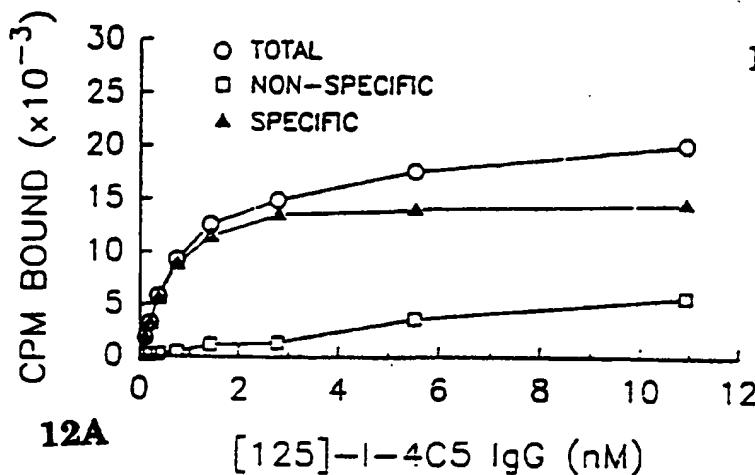
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60	70	80	90	100	110	120
YWTRQDRDLE EPINFRLPEN RISKEKDVLW FRPTLLNDTG NYTCMLRNTT YCSKVAPPLE VVQKDSCFNS						
130	140	150	160	170	180	190
AMRFPVHKMY IEHGIHKITC PNVDGYFPSS VKPSVTWYKG CTEIVDFHNV LPEGMNLSPP IPLVSNNGNY						
200	210	220	230	240	250	260
TCVVTYPEENG RLFHLTRTVT VKVVGSPKDA LPPQIYSPND RVVYEKEPGE ELVIPCKVYP SFIMDSHNEV						
270	280	290	300	310	320	330
WWTIDGKKPD DVTVDITINE SVSYSSTEDE TRTQILSIKK VTPEDLRRNY VCHARNTKGE AEQAAKVKQK						
340	350	360	370	380	390	400
VIPPRTYVEL ACGFGATVFL VVVLIVVYHV YWLEMVLFYR AHFGTDETIL DGKEYDIYVS YARNVEEEEF						
410	420	430	440	450	460	470
VLLTLRGVILE NEFGYKLCIF DRDSLPGGIV TDETLSFIQK SRRLLVVLSP NYVLOQGTQAL LELKAGLENM						
480	490	500	510	520	530	540
ASRGNINVIL VQYKAVKDMK VKELKRAKTV LTVIKWKGEK SKYPQGRFWK QLQVAMPVKK SPRWSSNDKQ						
550 GLSYSSLKNV						

14/23

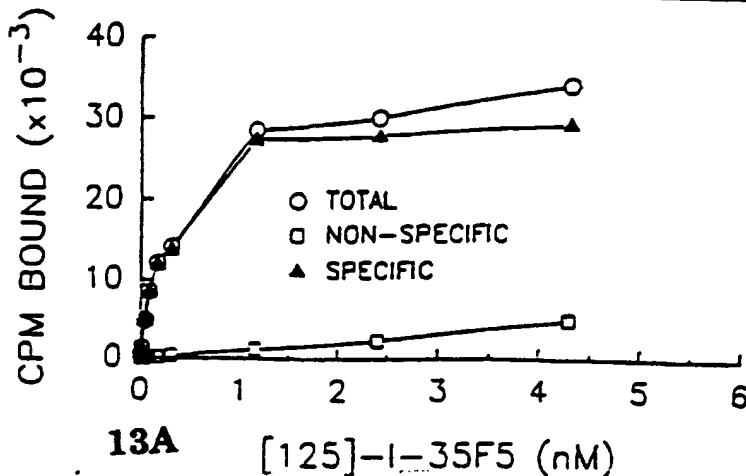
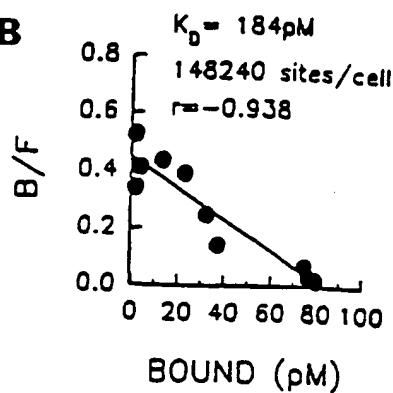
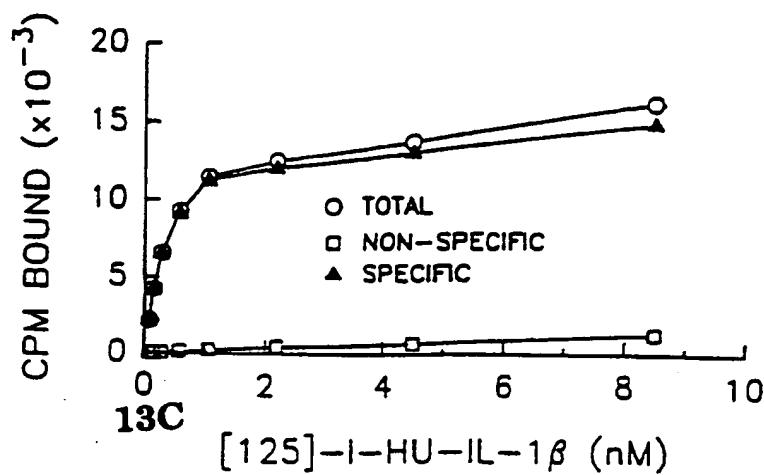
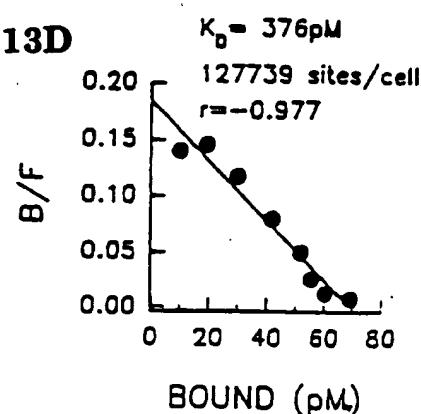
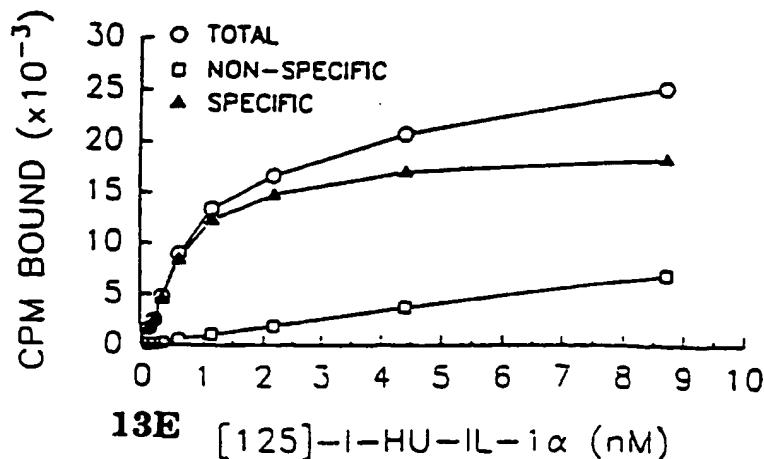
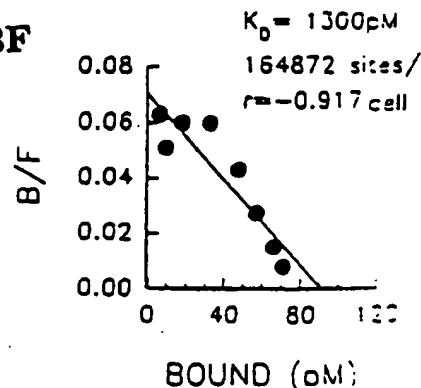
Fig. 11



15/23
[¹²⁵I]-I-4C5 IgG BINDING TO COS(AcP) CELLS

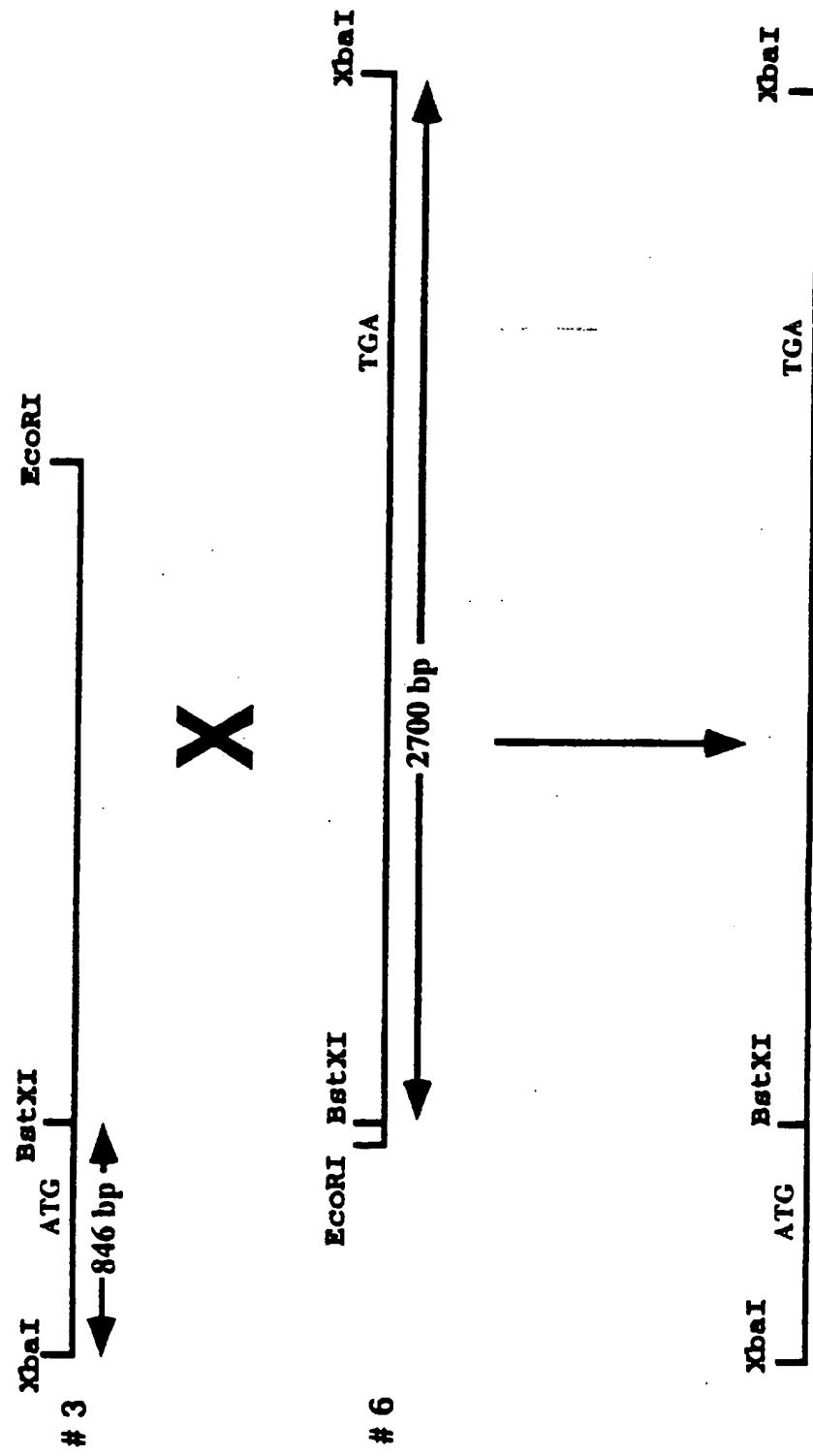
Fig. 12

16/23

Fig. 13[125]-I-35F5 IgG BINDING TO COS(MU-IL-1R) CELLS**13B**[125]-I-HU-IL-1β BINDING TO COS(MU-IL-1R) CELLS**13D**[125]-I-HU-IL-1α BINDING TO COS(MU-IL-1R) CELLS**13F**

17/23

Fig. 14



18/23

Fig. 15

10 20 30 40 50 60 70
 ATGACACTTC TGTGGTGTGT AGTGAGTC TACTTTATG GAATCCTGCA AAGTGATGCC TCAGAACCGCT
 TACTGTGAAG ACACCACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCACTACGG AGTCTTGCAG

 80 90 100 110 120 130 140
 GCGATGACTG GGGACTAGAC ACCATGAGGC AAATCCAAGT GTTGAAGAT GAGCCAGCTC GCATCAAGTG
 CGCTACTGAC CCCTGATCTG TGGTACTCCG TTTAGGTTCA CAAACTTCTA CTCGGTCGAG CGTAGTTCAC

 150 160 170 180 190 200 210
 CCCACTCTT GAACACTTCT TGAAATTCAA CTACAGCACA GCCCATTCAAG CTGGCCCTAC TCTGATCTGG
 GGGTGAGAAA CTTGTGAAGA ACTTTAAGTT GATGTCGTGT CGGGTAAGTC GACCGGAATG AGACTAGACC

 220 230 240 250 260 270 280
 TATTGGACTA GGCAGGACCG GGACCTTGAG GAGCCAATTA ACTTCCGCCT CCCCGAGAAC CGCATTAGTA
 ATAACCTGAT CCGTCCTGGC CCTGGAACTC CTCGGTTAAT TGAAGGCGGA GGGGCTTTG GCGTAATCAT

 290 300 310 320 330 340 350
 AGGAGAAAAGA TGTGCTGTGG TTCCGGCCCA CTCTCCTCAA TGACACTGGC AACTATACT GCATGTTAAG
 TCCTCTTCT ACACGACACC AAGGCCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTC

 360 370 380 390 400 410 420
 GAACACTACA TATTGCAGCA AAGTTGCATT TCCCTTGGAA GTTGTCAAAG AAGACAGCTG TTTCAATTCC
 CTTGTGATGT ATAACGTCGT TTCAACGTAA AGGGAACCTT CAACAAGTTT TTCTGTCGAC AAAGTTAAGG

 430 440 450 460 470 480 490
 CCCATGAAAC TCCCAGTGCA TAAACTGTAT ATAGAATATG GCATTCAAGAG GATCACTTGT CCAAATGTAG
 GGGTACTTTG AGGGTCACGT ATTTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA GGTTTACATC

 500 510 520 530 540 550 560
 ATGGATATTT TCCTTCCAGT GTCAAACCGA CTATCACTTG GTATATGGGC TGTTATAAAA TACAGAATT
 TACCTATAAAA AGGAAGGTCA CAGTTGGCT GATAGTGAAC CATATAACCG ACAATATTTT ATGTCTTAAA

 570 580 590 600 610 620 630
 TAATAATGTA ATACCCGAAG GTATGAACCTT GAGTTTCCCTC ATTGCCTTAA TTTCAAATAA TGGAAATTAC
 ATTATTACAT TATGGGCTTC CATACTTGAA CTCAAAGGAG TAACGGAATT AAAGTTTAACT ACCTTTAATG

 640 650 660 670 680 690 700
 ACATGTGTTG TTACATATCC AGAAAATGGA CGTACGTTTC ATCTCACCAAG GACTCTGACT GTAAAGGTAG
 TGTACACAAAC AATGTATAGG TCTTTTACCT GCATGCAAAG TAGAGTGGTC CTGAGACTGA CATTTCACATC

 710 720 730 740 750 760 770
 TAGGCTCTCC AAAAATGCA GTGCCCTCTG TGATCCATTC ACCTAATGAT CATGTGGTCT ATGAGAAAGA
 ATCCGAGAGG TTTTTTACGT CACGGGGGAC ACTAGGTAAAG TGGATTACTA GTACACCAGA TACTCTTCT

 780 790 800 810 820 830 840
 ACCAGGAGAG GAGCTACTCA TTCCCTGTAC GGTCTATTT AGTTTCTGA TGGATTCTCG CAAAGGGTT
 TGGCCTCTC CTCGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA

 850 860 870 880 890 900 910
 TGGTGGACCA TTGATGGAAA AAAACCTGAT GACATCACTA TTGATGTCAAC CATTAAACGAA AGTATAAGTC
 ACCACCTGGT AACTACCTT TTTGGACTA CTGTAGTGTAACTACAGTG GTAATTGCTT TCATATTCAAG

19/23

Fig. 15 cont.

920 930 940 950 960 970 980
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 TATCATCTTG TCTTCTACTT TGTTCTTGAG TCTAAAACTC GTAGTTCTTT CAATGGAGAC TCCTAGAGTT

 990 1000 1010 1020 1030 1040 1050
 GCGCAGCTAT GTCGTGTCATG CTAGAAGTGC CAAAGGCGAA GTGCCAAAG CAGCCAAGGT GACGCAGAAA
 CGCGTCGATA CAGACAGTAC GATCTTCACG GTTCCGCTT CAACGGTTTC GTCGGTCCA CTGCGTCTTT

 1060 1070 1080 1090 1100 1110 1120
 GTGCCAGCTC CAAGATAACAC AGTGGAACTG GCTTGTGGTT TTGGAGCCAC AGTCCCTGCTA GTGGTGATTC
 CACGGTCGAG TTCTATGTG TCACCTTGAC CGAACACCAA AACCTCGGTG TCAGGACGAT CACCACTAAG

 1130 1140 1150 1160 1170 1180 1190
 TCATTGTTGT TTACCATGTT TACTGGCTAG AGATGGTCCT ATTTTACCGG GCTCATTGTT GAACAGATGA
 AGTAACAACA AATGGTACAA ATGACCGATC TCTACCAGGA TAAAATGGCC CGAGTAAAC CTTGTCCTACT

 1200 1210 1220 1230 1240 1250 1260
 AACCATTTA GATGGAAAAG AGTATGATAT TTATGTATCC TATGCAAGGA ATGCCGAAGA AGAAGAATT
 TTGGTAAAT CTACCTTTTC TCATACTATA AATACATAGG ATACGTTCTT TACGCCCTCT TCTTCTTAA

 1270 1280 1290 1300 1310 1320 1330
 GTTTTACTGA CCCTCCGTGG AGTTTTGGAG AATGAATTG GATACAAAGCT GTGCATCTTT GACCGAGAC
 CAAAATGACT GGGAGGCACC TCAAAACCTC TTACTTAAAC CTATGTTCGA CACGTAGAAA CTGGCTCTGT

 1340 1350 1360 1370 1380 1390 1400
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 CAGACGGACC CCCTAACAG TGTCTACTCT GAAACTCGAA GTAAGTCTTT TCGTCTGCAG AGGACCAACA

 1410 1420 1430 1440 1450 1460 1470
 TCTAAGCCCC AACTACGTGC TCCAGGGAAC CCAAGCCCTC CTGGAGCTCA AGGCTGGCT AGAAAAATATG
 AGATTGGGG TTGATGCACG AGGTCCCTTG GGTCGGGAG GACCTCGAGT TCCGACCGGA TCTTTTATAC

 1480 1490 1500 1510 1520 1530 1540
 GGCTCTCGGG GCAACATCAA CGTCATTTA GTACAGTACA AAGCTGTGAA GGAAACGAAG GTGAAGAGAC
 CCGAGAGCCC CGTTGTAGTT GCAGTAAAT CATGTATGT TTCGACACTT CCTTGTCTC CACTTTCTCG

 1550 1560 1570 1580 1590 1600 1610
 TGAAGAGGGC TAAGACGGTG CTCACGGTCA TTAATGGAA AGGGGAAAAA TCCAAAGTATC CACAGGGCAG
 ACTTCTCCCG ATTCTGCCAC GAGTGCCAGT AATTACCTT TCCCCCTTTT AGGTTCATAG GTGTCCCGTC

 1620 1630 1640 1650 1660 1670 1680
 GTTCTGGAAAG CAGCTGCAGG TGGCCATGCC AGTGAAGAAA AGTCCCAGGC GGTCTAGCAG TGATGAGCAG
 CAAGACCTTC GTCGACGTCC ACCGGTACGG TCACTTCTT TCAGGGTCCG CCAGATCGTC ACTACTCGTC

 1690 1700 1710
 GGCCTCTCGT ATTCTATCTT GAAAAATGTA TGA
 CGGGAGAGCA TAAGTAGAAA CTTTTACAT ACT

20/23

Fig. 16

-20 -10 -1 1 10 20 30 40 50
MTLLNCVVS L YFYGILQSDA SERCDDWGLD TMHQIQVFED EPARIKCPLF EHFLKFNYST AHSAGLTLIW

60 70 80 90 100 110 120
YWTRQDRDLE EPINFRLPEN RISKEKDVLW FRPTLLNDTG NYTCMLRNTT YCSKVAFFPLE VVQKDSCFNS

130 140 150 160 170 180 190
PMKLPVHKLY IEYGIQRITC PNVDGYFPSS VKPTITWYMG CYKIQNFNNV IPEGRNLSFL IALISNNNGNY

200 210 220 230 240 250 260
TCVVTYPEENG RTFHILTRLT VKVVGSPKNA VPPVIHSPND HVVYEKEPGE ELLIPCTVYF SFLMDSRNEV

270 280 290 300 310 320 330
NWTIDGKKPD DITIDVTINE SISHSRTEDE TRTQILSIKK VTSEDLKRSY VCHARSAKGE VAKAAKVVTQK

340 350 360 370 380 390 400
VPAPRYTVEL ACGFGATVLL VVILIVYYHV YWLEMVLFYR AHFGTDETIL DGKEYDIYVS YARNAEEEF

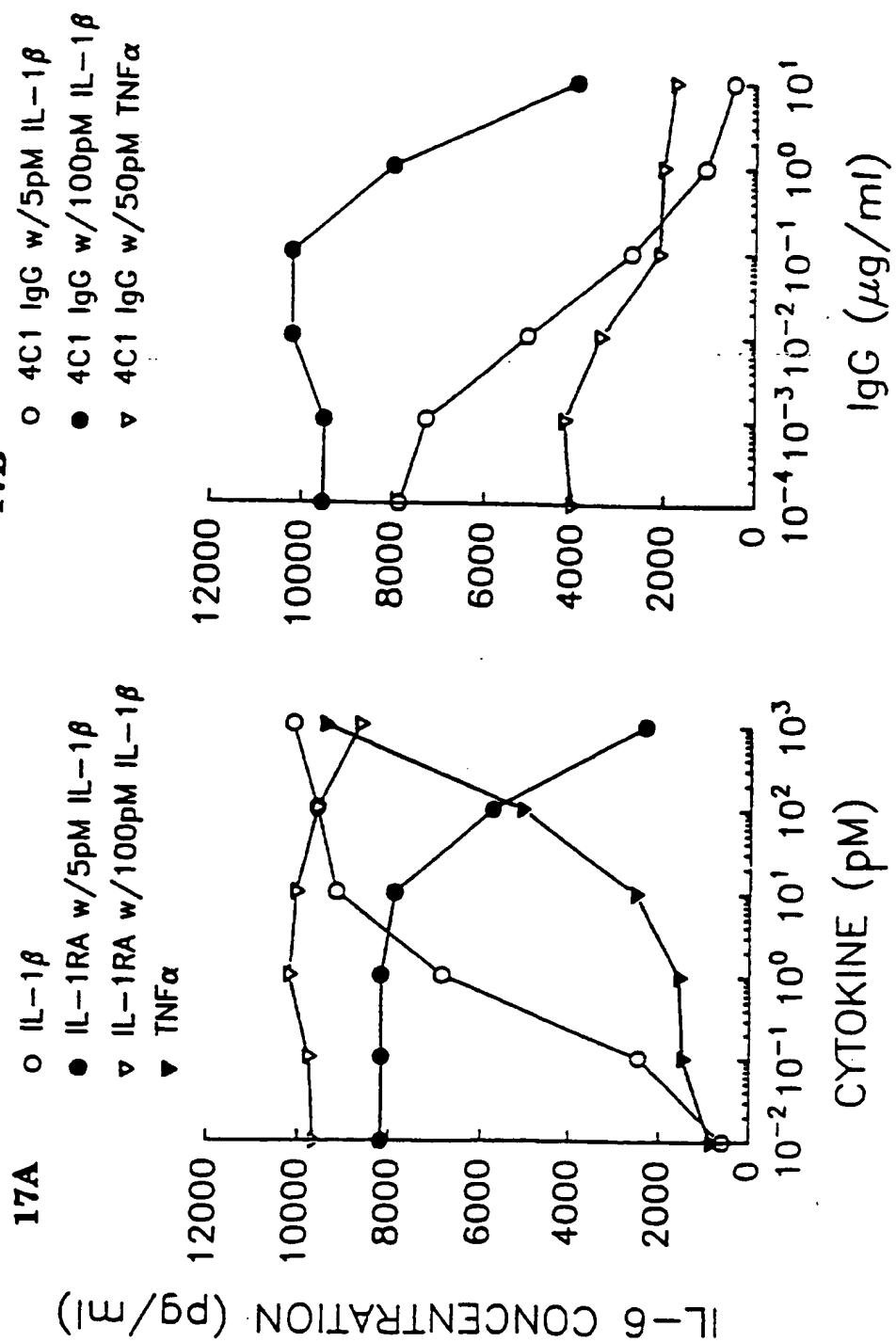
410 420 430 440 450 460 470
VLLTLRGVLE NEFGYKLCIF DRDSLPGGIV TDETLSFIQK SRRLLVVLSP NYVLOGTQAL LELKAGLENM

480 490 500 510 520 530 540
GSRGNIINVIL VQYKAVKETK VKEIJKRAKTV LTIVIKWKGEK SKYPQGRFWK QLOVAMPVIX SPERRSSSDEQ

550
GLSYSSLXNV

21/23

Fig. 17



22/23

Fig. 18

10 20 30 40 50 60 70
 ATGACACTTC TGTGGTGTGT AGTGACTCTC TACTTTATG GAACTCTGCC AAGTGATGCC TCAGAACGCT
 TACTGTGAAG ACACCAACACA TCACTCAGAG ATGAAAATAC CTTAGGACGT TTCACTACGG AGTCTTGGCA

 80 90 100 110 120 130 140
 CGCATGACTG GGGACTAGAC ACCATGAGGC AAATCCAAGT GTTGAAGAT GAGCCAGCTC GCATCAAGTG
 CGCTACTGAC CCCTGATCTG TGGTACTCCG TTAGGTICA CAAACTCTA CTCGGTCGAG CGTAGTTAC

 150 160 170 180 190 200 210
 CCCACTCTT GAACACTCT TGAAATTCAA CTACAGCACA GCTCCATTAG CTGGCCTTAC TCTGATCTGG
 GGGTGAGAAA CTGTGAAGA ACTTTAAGTT GATGTCGTGT CGGGTAAGTC GACCGGAATG AGACTAGACC

 220 230 240 250 260 270 280
 TATTGGACTA GGCAGGACCG GGACCTTGAG GAGCCATTAA ACTTCCGCT CCCCCGAGAAC CGCATTAGTA
 ATAACCTGAT CCGTCTTGGC CCTGGAACTC CTCGGTTAAI TGAAGGCGGA GGGGCTCTTG CGGTAAATCAT

 290 300 310 320 330 340 350
 AGGAGAGAAGA TGTGCTGTGG TTCCGGCCCA CTCTCTCAA TGACACTGGC AACTATACTT GCATGTTAAG
 TCTCTTTCT ACACGACACC AAGGCGGGT GAGAGGAGTT ACTGTGACCG TTGATATGGA CGTACAATTTC

 360 370 380 390 400 410 420
 GAACACTACA TATTGCAAGCA AAGTTGCAATT TCCCTTGGAA GTTGTICAAA AAGACAGCTG TTCAATTCC
 CTGTGAIGT ATAACGTGTT TTCAACGTAAGGGAAACCTT CAACAAGTTT TTCTGTCGAC AAAGTTAAGG

 430 440 450 460 470 480 490
 CCCATGAAAC TCCCAGTCCA TAAACTGTAT ATAGAAATAG GCATTCAGAG GATCACTTGT CCAAATGCTAG
 GGGTACTTTG AGGGTCACGT ATTGACATA TATCTTATAC CGTAAGTCTC CTAGTGAACA GGTTCATAC

 500 510 520 530 540 550 560
 ATGGATATTT TCCCTCCAGT GTCAAACCGA CTATCACTTG GTATATGGGC GTTGTATAAAA TACAGAATT
 TACCTATAAA AGGAAGGTCA CAGTTGGCT GATAGTGAAC CATATAACCG ACAATATTTT ATGTCTTAAA

 570 580 590 600 610 620 630
 TAATAATGTA ATACCCGAAG GTATGAACTT GAGTTTCTC ATTGCCTTAA TTCAAAATAA TGGAAATTAC
 ATTATTACAT TAIGGGCTTC CATACTGAA CTCAAAAGGAG TAACGGAAATT AAAGTTTAAIT ACCTTTAATG

 640 650 660 670 680 690 700
 ACATGTGTTG TTACATATCC AGAAAATGGA CGTACGTTTC ATCTCACCG GACTCTGACT GTAAAGGTAG
 TGTACACAAAC AAATGATAGG TCTTTTACCT GCATGCAAAG TAGAGTGGTC CTGAGACTGA CATTCCATC

 710 720 730 740 750 760 770
 TAGGCTCTCC AAAAATGCA GTGCCCCCTG TGATCCATTC ACCTAATGAT CATGTTGCT ATGAGAAAAGA
 ATCCGAGAGG TTGTTTACGT CACGGGGAC ACTAGGTAAG TGGATTACTA GTACACCAGA TACTCTTCT

 780 790 800 810 820 830 840
 ACCAGGAGAG GAGCTACTCA TTCCCTGTAC GGTCTTATTT AGTTTCTGA TGGATCTCG CAATGAGGT
 TGGCTCTC CTGGATGAGT AAGGGACATG CCAGATAAAA TCAAAAGACT ACCTAAGAGC GTTACTCCAA

 850 860 870 880 890 900 910
 TGGTGGACCA TIGATGAAA AAAACCTGAT GACATCACTA TTGAIGTCAC CATTAAAGCA AGTATAAGTC
 ACCACCTGGT AACTACCTT TTTGGACTA CTGTAATGAT AACTACAGTG GTAAATGCTT TCATATTCA
 920 930 940 950 960 970 980
 ATAGTAGAAC AGAAAGATGAA ACAAGAACTC AGATTTGAG CATCAAGAAA GTTACCTCTG AGGATCTCAA
 TATCATCTTG TCTTCTACTT TGTCTTGTAG TCTAAAACTC GTACTTCTT CAATGGAGAC TCCTAGAGTT

 990 1000 1010 1020 1030 1040 1050
 GCGCAGCTAT GTCTGTCTAG CTAGAAGTGC CAAACCGAA GTTGCCTAAG CAGCCAGGT GACCCAGAAA
 CGCGTCGATA CAGACAGTAC GATCTTCACG GTTCCGCTT CAACGGTTTC GTCGGTCTT

 1060 1070 1077
 GTGCCAGCTC CAAGATAACAC ACTGGAA
 CACGGTCGAG GTTCTATGTC TCACCTT

23/23

Fig. 19

-20 -10 -1 1 10 20 30 40 50
MTLLWCVVSL YFYGILQSSDA SERCDDWGLD TMRQIQVNFED EPARIKCPLF EHFLKFNYST AHSAGLTLIW
60 70 80 90 100 110 120
YWTRQDRDLE EPINFRIPEN RISKEKDVLW FRPTLINDTG NYTCMLRNTT YCSKVAFPLE VVQKDSCFNS
130 140 150 160 170 180 190
PMKLPVHKLY IEXYGIQRITC PNVDGYFPSS VKPTITWYMG CYKIQNFIINN IPEGMNLSSL IALISNNNGNY
200 210 220 230 240 250 260
TCVVTYTFENG RTFHLTRLT VRVVGSPRNA VPPVVIHSPND HVVIEKEPGE ELLIPCTVYF SFLMDSRNEV
270 280 290 300 310 320 330
WWTIDGKKPD DIITIDVTINE SISHSRTEDE TRTQIILSIKK VTSEDLKRSY VCHARSAKGE VAKAAKVVTQK
339
VPAPRYTE

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/00181

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6	C12N15/12	C07K14/715	C07K16/28	A61K38/17	C12N5/20
	A61K39/44				

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6	C07K
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>JOURNAL OF CELLULAR BIOCHEMISTRY SUPPL 0, no. 18B, 1994, page 217 XP002007812</p> <p>S. GREENFEDER ET AL: "Expression cloning of a cDNA encoding a novel murine Interleukin 1 receptor accessory protein" see abstract</p> <p>& KEYSTONE SYMPOSIUM ON TRANSMEMBRANE SIGNAL TRANSDUCTION :STRUCTURE, MECHANISMS, REGULATION OF EVOLUTION, 6 - 13 February 1994, KEYSTONE, COLORADO, USA.,</p> <p>---</p> <p>-/-</p>	1,2, 11-15, 26,28

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8 July 1996	12.07.96
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Le Cornec, N

INTERNATIONAL SEARCH REPORT

International Application No
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C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY PART II, vol. 150, no. 8, 15 April 1993, BALTIMORE US, page 46A XP002007498 G. D. POWERS ET AL: "Differential inhibition of murine B cell responses to IL-1 by monoclonal antibodies specific for type 1 and type 2 IL-1 receptors and a putative IL-1 receptor accessory protein" cited in the application	14,15, 21-23
X	& JOINT MEETING OF THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS AND THE CLINICAL IMMUNOLOGY SOCIETY, 21 - 25 May 1993, DENVER, COLORADO., see abstract 250 ---	27,28
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 23, 9 June 1995, MD US, pages 13757-13765, XP002007499 S.A. GREENFEDER ET AL: "Molecular cloning and characterization of a second subunit of the Interleukin 1 receptor complex" see the whole document -----	1-3,5, 11-15, 26-28

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